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Index number

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Going.

For Rx control of VVC

Going.

Gone.

In vulvovaginal candidiasis, **the proven efficacy of 3-day therapy[*] with FEMSTAT Prefill means a microbiological cure in 95% of patients.[1†]**

[1] Droegemueller W, Adamson DG, Brown D, et al. Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64:530-534.

[*] In pregnant patients (second and third trimesters only), therapy should be extended to six days.

[†] Microbiological cure in 95% of patients (n = 126) eight days following FEMSTAT 3-day therapy.

[‡] Micrographs on file, Syntex Laboratories, Inc.

Please see next page for brief summary of prescribing information.

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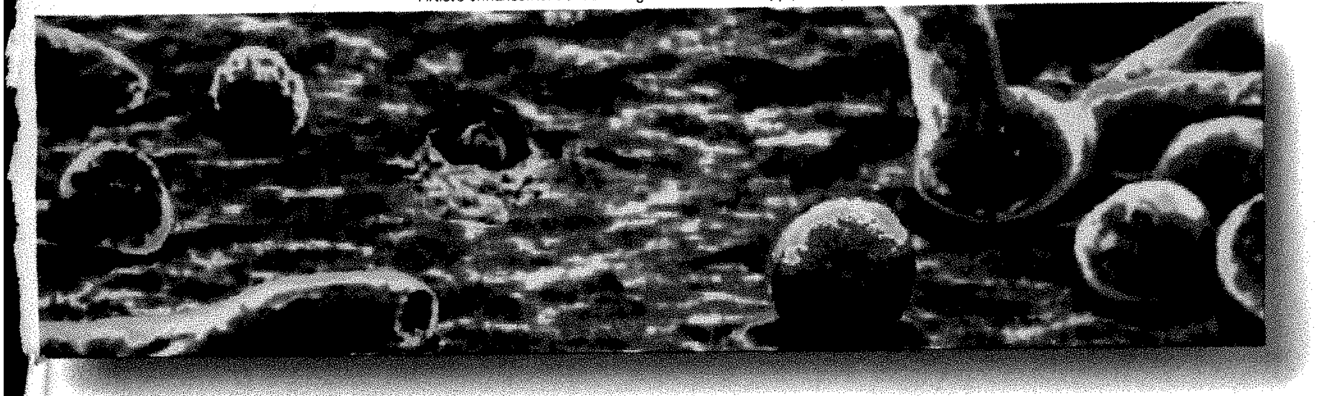
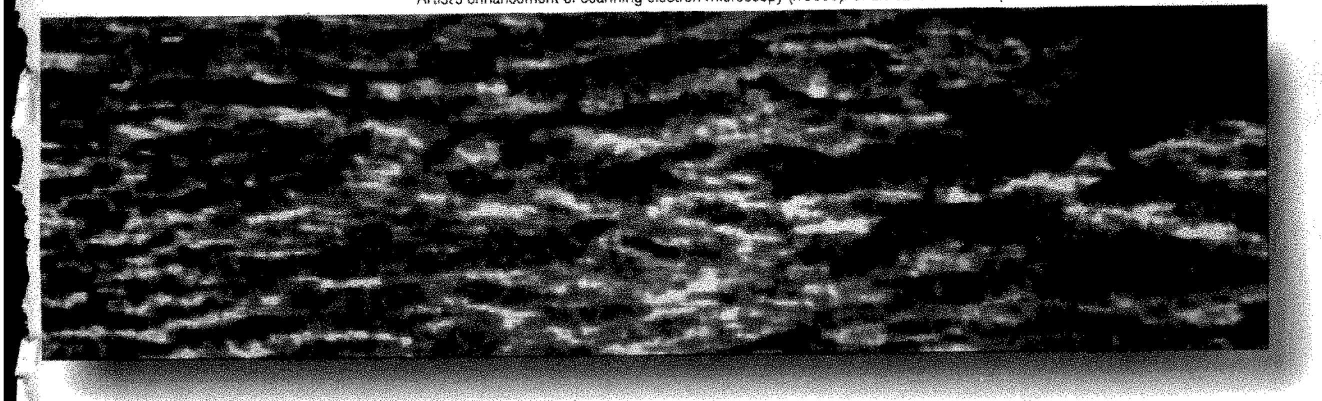
A T

Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 24 hours posttreatment with butoconazole nitrate.

Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 72 hours posttreatment with butoconazole nitrate.



Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* one week posttreatment with butoconazole nitrate.



P 24,298

(36)

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FEMSTAT® PREFILL

(butoconazole nitrate) VAGINAL CREAM 2%

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Committed to the OB/GYN and women's healthcare

FEMSTAT PREFILL
(butoconazole nitrate) VAGINAL CREAM 2%

BRIEF SUMMARY

INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

CAUTION:
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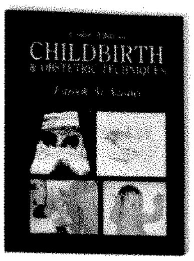

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- Full color photographs depict all aspects of childbirth, with line drawings used to explain movement of the baby.
- Covers prenatal care and management of labor.

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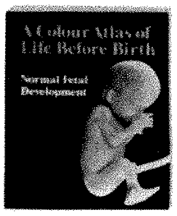

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American Journal of Obstetrics and Gynecology

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June
Part 1
1991

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TRANSACTIONS OF THE FIFTY-EIGHTH ANNUAL MEETING OF THE CENTRAL ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Winter hath hope of spring 1401
L. Russell Malinak, MD
Houston, Texas

The mammary stimulation test—A predictor of preterm delivery? 1409
Robert D. Eden, MD, Robert J. Sokol, MD, Yoram Sorokin, MD, Helen J. Cook, RNC, MSN, Gail Sheeran, RNC, and Lawrence Chik, PhD
Detroit, Michigan

The mammary stimulation test appears to improve the prediction of preterm delivery in patients with risk factors for premature delivery.

A randomized trial evaluating leuprolide acetate before hysterectomy as treatment for leiomyomas 1420
Thomas G. Stovall, MD, Frank W. Ling, MD, Louie C. Henry, MD, and Marie R. Woodruff, RN
Memphis, Tennessee

A 2-month treatment course of leuprolide acetate before hysterectomy increases the likelihood that the patient can undergo vaginal hysterectomy.

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Vol. 164, No. 6, Part 1, June 1991. The American Journal of Obstetrics and Gynecology (ISSN 0002-9378) is published monthly (six issues per volume, two volumes per year) by Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318. Second-class postage paid at St. Louis, Missouri, and additional mailing offices. POSTMASTER: Send address changes to American Journal of Obstetrics and Gynecology, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318, (314) 453-4351. Annual subscription rates for 1991: domestic, $86.00 for individuals and $150.00 for institutions. Printed in the U.S.A. Copyright © 1991 by Mosby–Year Book, Inc.

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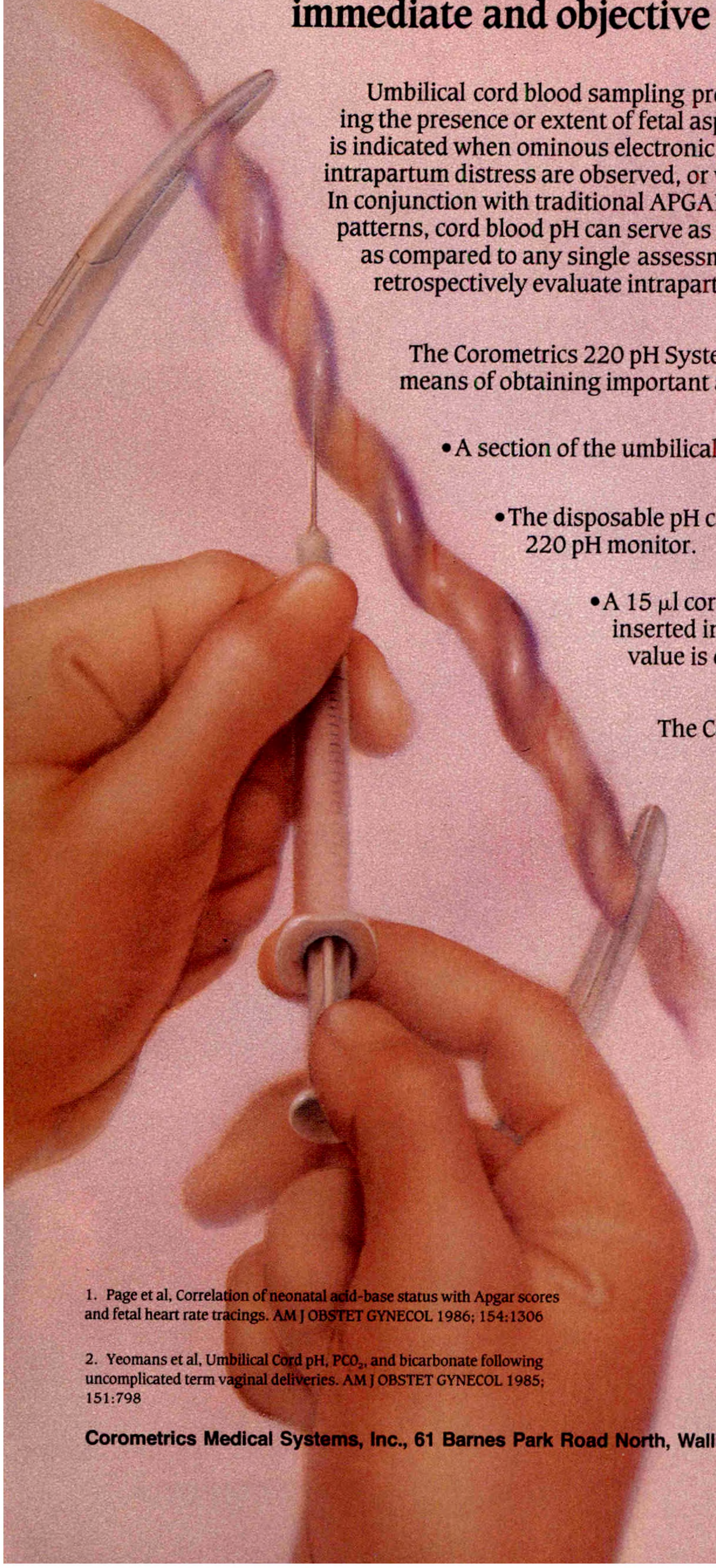

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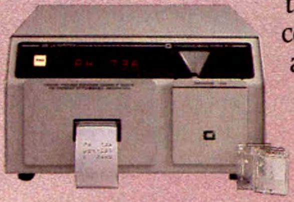

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1. Page et al, Correlation of neonatal acid-base status with Apgar scores and fetal heart rate tracings. AM J OBSTET GYNECOL 1986; 154:1306

2. Yeomans et al, Umbilical Cord pH, PCO_2, and bicarbonate following uncomplicated term vaginal deliveries. AM J OBSTET GYNECOL 1985; 151:798

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Emanuel P. Gaziano, MD, G. Eric Knox, MD, Richard P. Bendel, MD, Steven Calvin, MD, and Deb Brandt, RDMS

Minneapolis, Minnesota

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Dev Maulik, MD, PhD, Prasad Yarlagadda, PhD, James P. Youngblood, MD, and Pamela Ciston, RDMS

Kansas City, Missouri

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Gerald F. Joseph, Jr., MD, Charles M. Stedman, MD, and Alfred G. Robichaux, MD

New Orleans, Louisiana

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§Individual subscriptions and all student-rate subscriptions must be in the names of, billed to, and paid by individuals. All student-rate requests must indicate training status and name of institution.

Subscriptions may begin at any time.

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Indianapolis, Indiana

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Ronald T. Burkman, MD, William R. Bell, MD, Howard A. Zacur, MD, PhD, and Allyn W. Kimball, PhD

Detroit, Michigan, and Baltimore, Maryland

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Richard Reid, MD, Mitchell D. Greenberg, MD, Attila Lorincz, PhD, A. Bennett Jenson, MD, C.R. Laverty, MD, Mujtaba Husain, MD, Yahya Daoud, MA, Barina Zado, MD, Thomas White, MD, David Cantor, MD, and Milton Goldrath, MD

Detroit and Kalamazoo, Michigan, Silver Spring, Maryland, Washington, D.C., and Eastwood, Australia

A new screening model is advanced in which the added initial cost of extra testing is rewarded by almost optimal sensitivity and specificity; major reductions in the expense of unnecessary patient recall for diagnostic workup and empiric therapy would yield better patient safety at a lower cost.

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Gregory P. Sutton, MD, Margaret R. Miser, MD, Frederick B. Stehman, MD, Katherine Y. Look, MD, and Clarence E. Ehrlich, MD

Indianapolis, Indiana

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Isa Bernardini, MS, Mark I. Evans, MD, Kypros H. Nicolaides, MD, Demetrious L. Economides, MD, and William A. Gahl, MD, PhD

Bethesda, Maryland, Detroit, Michigan, and London, England

The fetal concentrating index (the in utero fetal/maternal ratio of six concentrated amino acids) can be used to detect growth-retarded fetuses prenatally.

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Cefotan arithmetic.

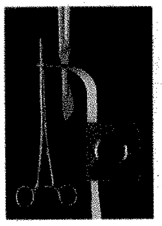
•CDC•
CENTERS FOR DISEASE CONTROL
RECOMMENDED
FOR PID[7]
CEFOTAN IV 2g, q12h + doxycycline IV or PO 100 mg, q12h

+ more cost-effective than cefoxitin[1,2]

− less frequent dosing than cefoxitin[3,4]

× several times the half-life of cefoxitin[5,6]

÷ single-dose prophylaxis/ twice-daily treatment



Count on

CEFOTAN® IM/IV
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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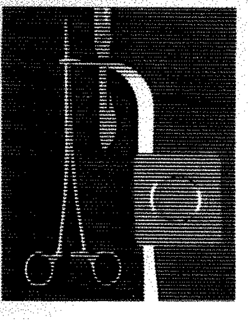

Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE

TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary tract infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri*, and *Morganella morganii*).

Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli*.

Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli*.

Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).

Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).

Bone and joint infections caused by *Staphylococcus aureus*.*

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes should also be considered.

PRECAUTIONS

GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

CEFOTAN® (cefotetan disodium)

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:}\quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:}\quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED

CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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Abbey B. Berenson, MD, Norma J. Stiglich, MD, Gregg S. Wilkinson, PhD, and Garland D. Anderson, MD
Galveston, Texas

A 20% prevalence of prior physical abuse was found in a pregnant population; physical abuse varied by race and was significantly associated with using drugs, alcohol, and tobacco.

The natural history of HELLP syndrome: Patterns of disease progression and regression 1500

James N. Martin, Jr., MD, Pamela G. Blake, MSN, Kenneth G. Perry, Jr., MD, James F. McCaul, MD, L. Wayne Hess, MD, and Rick W. Martin, MD
Jackson, Mississippi

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C. Robert Stanhope, MD, Timothy O. Wilson, MD, William J. Utz, MD, Lynwood H. Smith, MD, and Peter C. O'Brien, PhD
Rochester, Minnesota

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Michael P. Hopkins, MD, and George W. Morley, MD
Ann Arbor, Michigan

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Albert J. Peters, DO, and Carolyn B. Coulam, MD
Indianapolis, Indiana

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Comparison of associated high-risk factors and perinatal outcome between symmetric and asymmetric fetal intrauterine growth retardation 1535

Chin-Chu Lin, MD, Shyr-Jou Su, MD, and L. Philip River, BA
Chicago, Illinois

Except for preeclampsia, no differences were seen in seventeen associated high-risk factors between symmetric and asymmetric IUGR, but the likelihood of preterm birth and neonatal morbidity was higher for symmetric IUGR infants.

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James M. Maharry

Whittier, California

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Seth L. Feigenbaum, MPH, MD, Mary C. Martin, MD, Charles B. Wilson, MD, and Robert B. Jaffe, MD

San Francisco, California

Pregnant or postpartum women with symptoms of a pituitary mass should be evaluated for lymphocytic adenohypophysitis and corticosteroid therapy considered before routine neurosurgical intervention.

Antibiotic treatment of tuboovarian abscess: Comparison of broad-spectrum β-lactam agents versus clindamycin-containing regimens 1556

Susan D. Reed, MD, Daniel V. Landers, MD, and Richard L. Sweet, MD

San Francisco, California

Broad-spectrum β-lactam antibiotics plus doxycycline are as effective as clindamycin-containing regimens in the treatment of tuboovarian abscesses (overall success rate, 75%).

Antepartum testing in the hypertensive patient: When to begin 1563

Richard A. Pircon, MD, David C. Lagrew, MD, Craig V. Towers, MD, Wendy L. Dorchester, MS, Steve E. Gocke, MD, and Roger K. Freeman, MD

Milwaukee, Wisconsin, Orange and Long Beach, California

When to start antepartum fetal surveillance in the hypertensive pregnant patient is presented.

The roles of ultrasonography and amniocentesis in evaluation of elevated maternal serum α-fetoprotein 1571

Karen K. Lindfors, MD, David P. Gorczyca, MD, Frederick W. Hanson, MD, Frances R. Tennant, PhD, John P. McGahan, MD, and Ann G. Peterson, MN

Sacramento, California

Ultrasonography and amniocentesis are complementary in the evaluation of an elevated maternal serum α-fetoprotein level; both are needed for accurate prenatal diagnosis.

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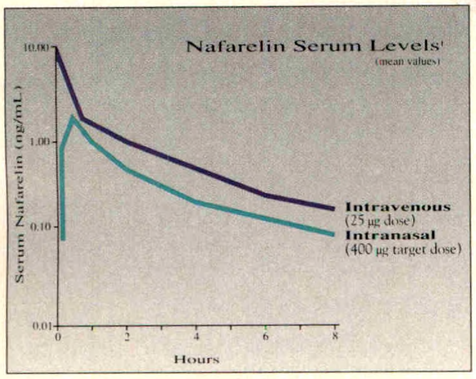
The Only GnRH* Agonist With The Dosing Distinction Of Intranasal Delivery

- Intranasal administration produces nafarelin serum levels comparable to I.V. dosing: In pharmacokinetic studies, SYNAREL was rapidly absorbed into systemic circulation following intranasal administration, with maximum serum concentrations achieved between 10 and 40 minutes.[1]



- B.I.D. controlled-dose intranasal administration suppresses serum estradiol to levels that result in outstanding clinical efficacy, while producing minimal impact on bone and a predictable hypoestrogenic profile.†
- Facilitates ease of patient management should allergic or other adverse events occur.
- Retreatment cannot be recommended since safety data for retreatment are not currently available.

1. Syntex document #829-222-A.

*Gonadotropin-releasing hormone analog.

†There is a small, largely reversible loss in bone density with 6-month treatment. Use of SYNAREL for longer than the recommended 6 months or in the presence of other known risk factors for decreased bone mineral content may cause additional bone loss.

Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a non-hormonal method of contraception when taking SYNAREL.

Please see adjacent page for brief summary of prescribing information.

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SYNAREL®

(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

Setting The Pace In Endometriosis Therapy

BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.
INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.
CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.
WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.
When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.
PRECAUTIONS Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. Patients missing successive doses may experience breakthrough bleeding.
2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients.
3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician.
4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients.
5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content.
6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption.
7. Retreatment cannot be recommended since safety data beyond 6 months are not available.
Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.
Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.
Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.
Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.
Pediatric Use: Safety and effectiveness in children have not been established.
ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.
Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.
Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.
Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.
Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.
Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.
OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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Robert C. Goodlin, MD

Denver, Colorado

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Mauricio I. Espinoza, MD, and Julian T. Parer, MD, PhD

San Francisco, California

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Kenneth A. Burry, MD, Laura H. Greenberg, MD, and Phillip E. Patton, MD

Portland, Oregon

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Lewis Shenker, MD, Kathryn L. Reed, MD, Caroline F. Anderson, RDMS, and Nydia A. Borjon, RDMS

Tucson, Arizona

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Philip G. Brooks, MD, Scott P. Serden, MD, and Irene Davos, MD

Los Angeles, California

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Martha F. Goetsch, MD

Portland, Oregon

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Lawrence D. Platt, MD, Dru E. Carlson, MD, Arnold L. Medearis, MD, and Catherine A. Walla, RN, MA, MN
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Val Davajan, MD, Oscar Kletzky, MD, Michael Vermesh, MD, and Debra J. Anderson, MD

Los Angeles and Torrance, California

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Duncan R. Neilson, Jr., MD, Richard N. Bolton, MD, Robert P. Prins, MD, and Carl Mark III, MD

Portland, Oregon

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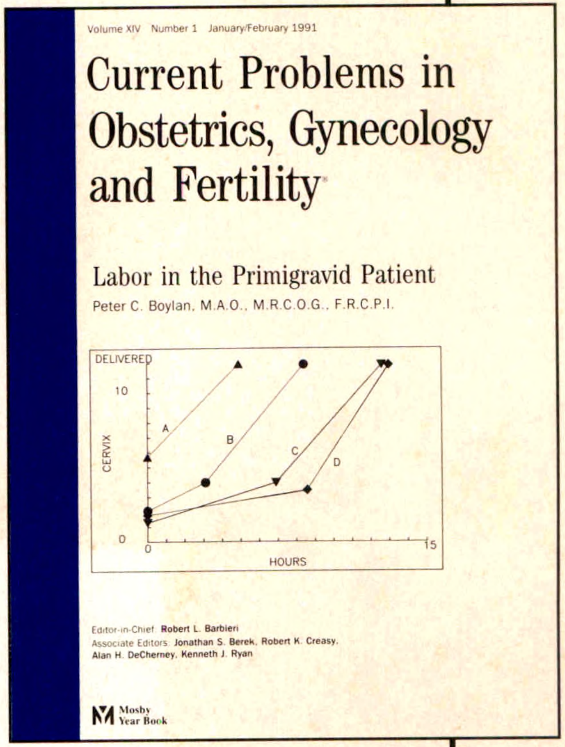
Volume XIV Number 1 January/February 1991

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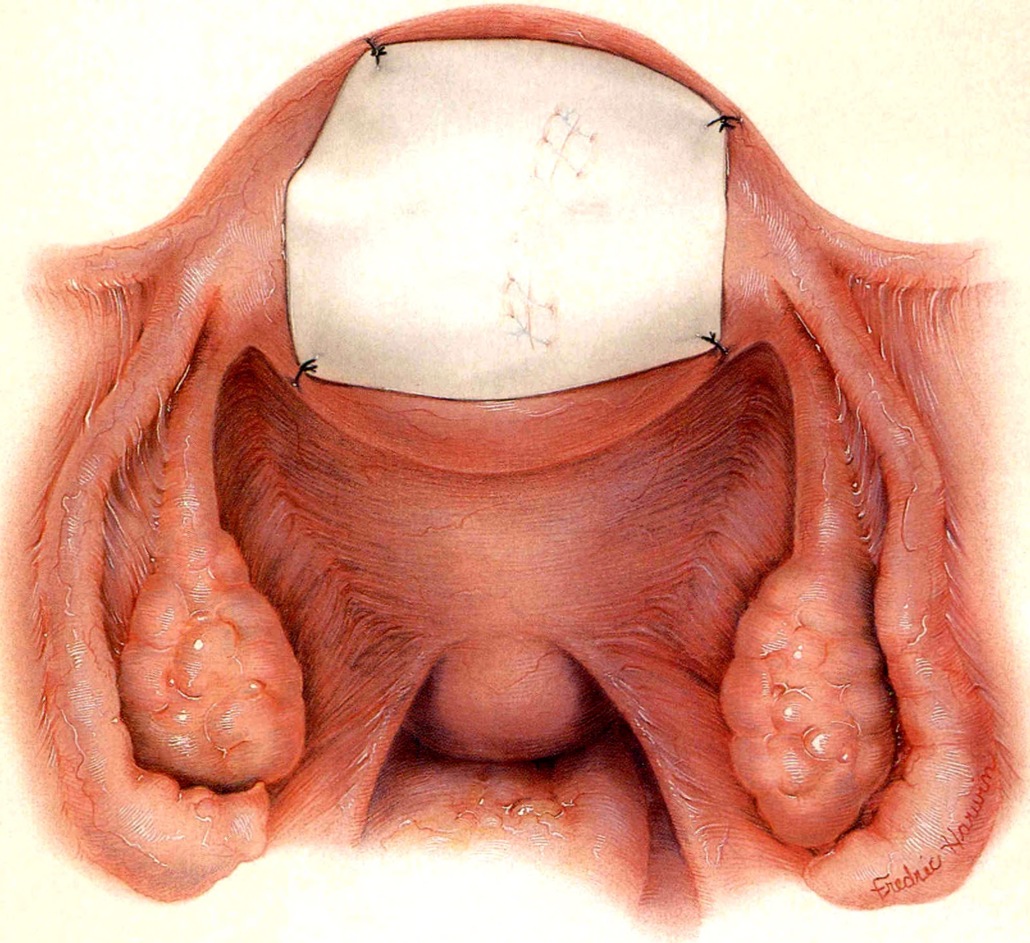

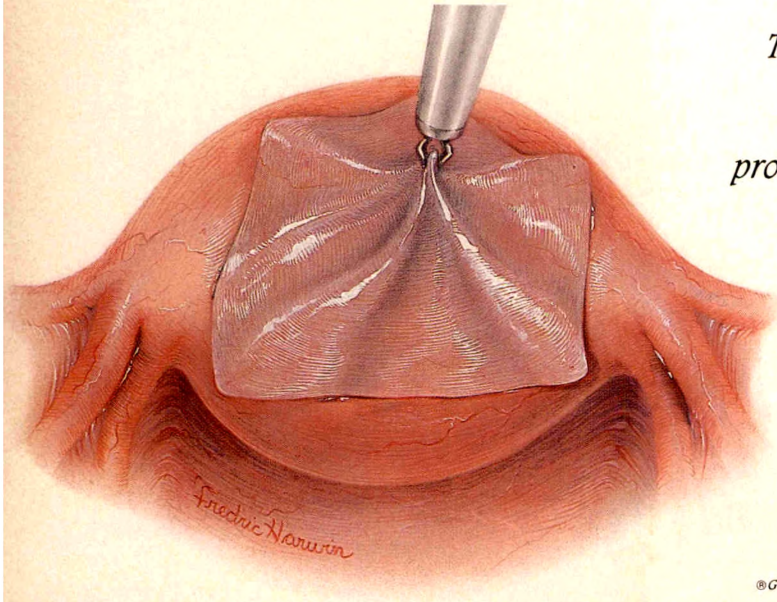

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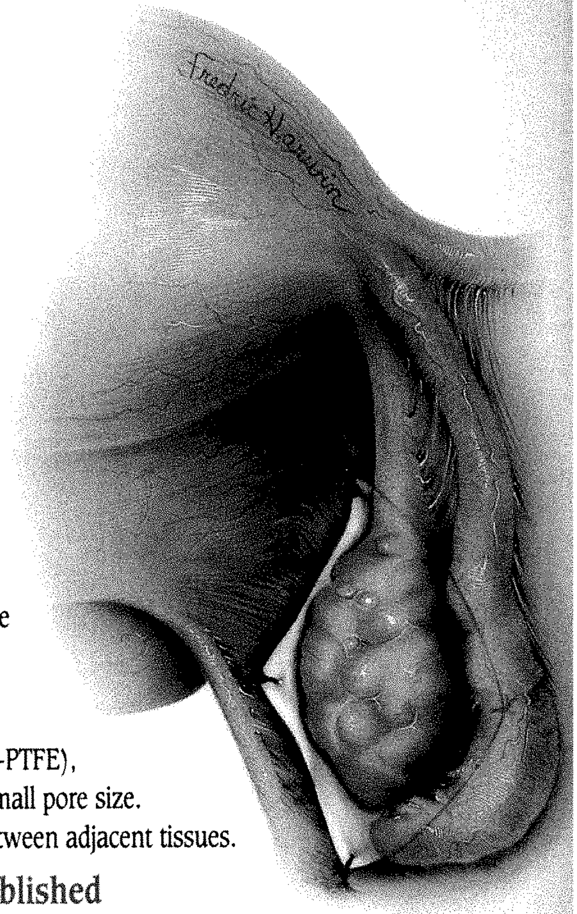

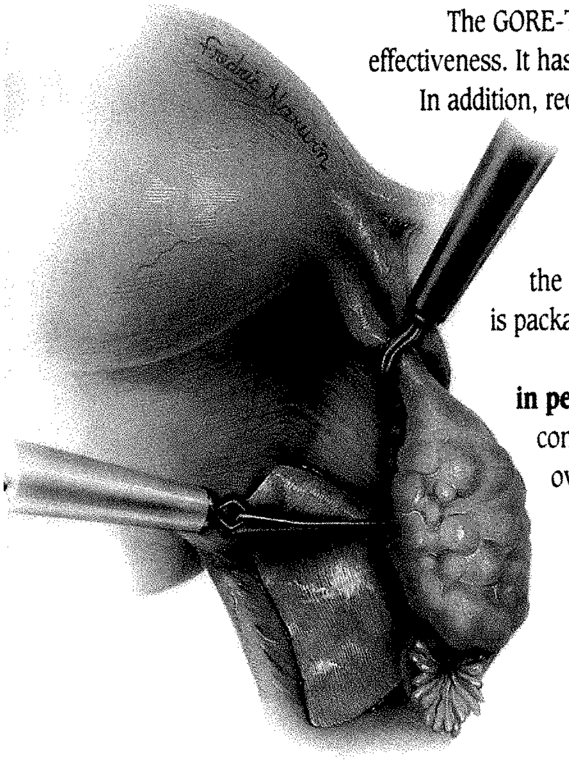

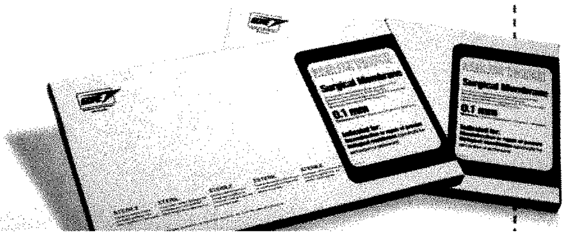

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Abstract page and key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

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In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

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With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

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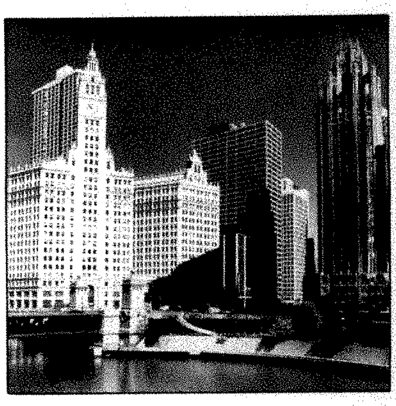

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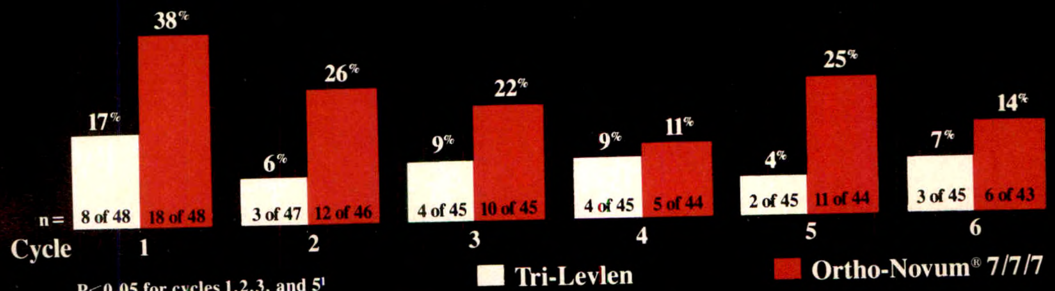

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Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemics, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's—but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

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Winter hath hope of spring*

Presidential address

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Houston, Texas

A harsh winter wind is blowing on American medicine today.

I use this metaphor to describe the relentless and escalating criticism of our medical care delivery system that has been occurring in the press and other media.

Much of the critical rhetoric has been expressed by those recommending a major restructuring of American medical care. As the volume of criticism grows, it is clear that the objective is to direct public opinion toward a centralized, national system for all patients and away from the private practice of medicine.

Virtually every area of medicine has been the subject of these critics' condemnation. With increasing frequency newspaper and magazine articles, television, and other media have projected a poor image of our profession. In many situations the criticism is unfounded or it is based on incomplete information or erroneous assumptions. I would like to explore one such aspect of American medicine.

Perinatal mortality has been the single most visible medical care issue attracting media attention in the United States during the last three decades.[1] Recent statistics rank the United States eighteenth behind the world leader, Japan. For many years the United States has ranked behind other developed nations; this situation has been called a barometer of our nation's health and has stimulated persistent discussion of the need to change our method of health care delivery.[2]

My interest in this problem began because of a personal experience in 1958. As a medical student I observed the death of an 18-year-old primigravid woman and her infant in utero in association with an eclamptic seizure. The seizure had begun while the patient was at home and continued until her death, which occurred shortly after she arrived at Charity Hospital in New Orleans, Louisiana, prior to any opportunity to initiate therapy. Having seen eclamptic patients successfully treated with good outcome for mother and baby, I was very frustrated by the tragic loss of these two young lives. I was even more frustrated to learn that the patient had not received any prenatal care, in spite of living within easy walking distance of Charity Hospital, where prenatal clinics were routinely and frequently held.

The impact of these deaths stimulated me to follow the scientific literature and media reporting on perinatal mortality through the 1960s, 1970s, and 1980s. There have been consistently negative media reports about, and overt criticism of, American medicine during this 30-year span.

A typical example from the 1960s was published in *Look* magazine. Mr. R.H. Berg[3] stated, "The state of our nation's health is bad. We rank far below less affluent countries. In preventing infant deaths, the United States stands tenth."

Such commentary did not escape the interest and political posturing of President Lyndon Johnson and the architects of his "Great Society."[4] They made persistent calls for so-called "socialized medicine," in line with their many other ideas about the remaking of American society.

The frequency and urgency of media statements calling for a major change in our medical care system have increased, as the rank of the United States in perinatal mortality rate has declined compared with rates in other industrialized countries.

Without critical analysis of the complex origins of

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From the phrase "not winter these hath hope of spring" in the poem, "The North Wind Came up Yesternight," Robert Bridges (1844-1930).

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our high perinatal mortality rate or, worse, completely ignoring some crucial issues related to the possible origins of this problem, media reports are currently railing at the medical profession and demanding a major overhaul in the way we take care of patients. As usual, politicians have taken the lead in these headlines.

Recent Texas Senator, now United States Congressman Craig Washington declared, "The state of our health care is just plain appalling." Since our country ranks seventeenth in infant mortality, he says, we must conclude that our health care system has to change.[5]

And, in decrying our high infant mortality rate, Reverend Jesse Jackson stated, "Our present system is not a health care system, it is a **sick** care system." He urged adoption of national health insurance.[6]

Recently, additional headlines suggested that our infant death rate represents a major deficiency in our medical care system. Shapiro, in *The San Francisco Examiner,* claimed that United States health is lagging, highlighting our high infant death rate. "Other countries think we're nuts for paying so much, getting so little," he said.[7] Humphrey Taylor, President of the Louis Harris Polling Organization, maintains we have the costliest, most disliked health care anywhere, because "people live longer and infant mortality rates are lower in other countries," among several other complaints.[8]

You may have noticed different rankings quoted in these news stories published at approximately the same time. They are based on data compiled in different years and drawn from different sources; thus some confusion exists. Also, methods of reporting perinatal mortality from different countries have been highly variable over the last three decades. Particularly in the earlier years, but even in recent times, the variations in data gathering or the **lack** of data disallow many commonly made comparisons.

Nevertheless, what is the scope of the problem in America? Currently, there are 40,000 infant deaths and 30,000 stillbirths per year in our country.[9]

A majority of scientific publications before the 1980s, which ranked countries according to perinatal mortality, used crude infant and neonatal mortality rates.[10]

There are problems with these comparisons. Comparisons of crude neonatal mortality rates cannot be used to determine effectiveness of prenatal or neonatal medical care, because of differences in birth weight distributions between countries. This is analogous to comparing crude death rates without standardizing for important variables such as age and sex.[11]

As the United States slipped progressively farther behind the countries of northern Europe in perinatal mortality in the 1970s and 1980s, peer-review publications began examining factors other than crude infant mortality rates. Variables such as socioeconomic status, maternal age, birth order, and, above all, birth weight were evaluated.

From a data base encompassing 1967 to 1978 in Norway, a country internationally recognized for having low infant mortality, Erickson and Bjerkedal[12] in 1982 noted that in industrialized countries most infant deaths occur in the neonatal period, the first month of life. And they, among others, convincingly demonstrated that neonatal mortality is much more powerfully affected by the birth weight of the infant than it is by any other variable.

Thus, rather than comparing crude neonatal death rates between two countries, they compared birth-weight-specific rates and found that babies of the same weight, on average, have lower death rates in the United States than they do in Norway. These results suggest that perinatal medical care is better in the United States than in Norway. This finding was confirmed by the work of Lee,[13] who compared several national neonatal mortality rates, after adjusting them for very low birth weight, and found similar results.

In an editorial, Paneth[14] stressed that infant mortality is closely tied to indices of social disadvantage. To connect this known association with the observations of Erickson and Bjerkedal, he further stressed that the strong association of social class with neonatal mortality is almost entirely due to its effect on the distribution of birth weights in populations. Put simply, "Poor mothers have small babies and small babies are at high risk of early death."[14]

Because of this fact, two components of neonatal and infant mortality rates must be studied: the distribution of birth weights and the mortality at each birth weight. The former reflects socioeconomic circumstances, although genetic differences in birth weight should not be ignored. The latter reflects the quality of medical care.[14]

Support for these concepts came quickly. David and Siegel[15] and McCormick[16] determined that the contribution of social-behavioral factors to the problem of neonatal mortality can be estimated by determining a population's birth-weight distribution.

Confirming the Norway findings in Sweden, Guyer et al.[11] demonstrated that crude death rates were lower in that country than in Massachusetts, but weight-specific neonatal survival rates were actually better in Massachusetts. The clear implication is that perinatal medical care is at least as good in Massachusetts as in Sweden, if not better. I recall no headlines in the popular press about this information.

Infant mortality means dead children; the emotional impact of this image is heartbreaking. There are few, if any, societies where the death of an infant is not thought of as a personal or family tragedy. Thus prevention of deaths in infants should be a societal imperative.[17]

To reduce the number of neonatal deaths in America, we must leave no stone unturned to learn the many origins of this devastating problem.

We must first concentrate on what is now certainly the most important origin of neonatal death—the societal and socioeconomic factors that result in low-birth-weight babies.

The medical and technical aspects of health care of neonates in America are outstanding and are continually improving.

Resnick et al.[18] have estimated the probability of survival of neonates at birth in 16,183 infants treated from 1980 to 1987 in the Perinatal Intensive Care Program of Florida. The survival curves for infants weighing >1500 gm converged to 95% survival. This is a remarkable record, and the likelihood of survival, even among very-low-birth-weight infants, is high, indicating the commendable level of American medical care. These results are second to none in the world.

Of historical interest is that by 1910 it had been established that the death rate among children was high in proportion to the social status of the population. This relationship was early recognized as a prominent feature of neonatal mortality[19]; however, it received little attention in the scientific literature and virtually no reporting in the popular press for many years. It is puzzling that such a basic concept was overlooked or put aside for such a long period of time.

Recent publications[1, 20] have studied the societal and socioeconomic factors that result in low-birth-weight babies; they have documented known factors and have speculated about others.

Five critical influences on the incidence of low birth weight are commonly discussed; these are: (1) adequacy of prenatal care, (2) smoking, (3) drug abuse, (4) infections, and (5) nutrition. Smoking and drug abuse are given prominence because they can be prevented and cessation will have immediate beneficial results for babies. Other influences include low level of education, single marital status, teenage pregnancy, race, and financial problems. Perhaps the work of Berenson et al.[21] presented at this meeting will result in the addition of physical abuse to this list.

The association of inadequate or absent prenatal care with low birth weight and consequent perinatal mortality is among the best-identified predictors of infant death. Too many poor Americans do not receive appropriate prenatal care.

The American College of Obstetricians and Gynecologists has taken a leadership role among United States organizations in an attempt to impress medical and social institutions with the urgent need to rectify this situation. Dr. Richard Schwarz,[22] current President-Elect of the College, in a 1989 editorial entitled, "Infant Mortality and Access to Care," pointed to the unenviable rank (seventeenth) of America among industrialized nations and stressed that financial and cultural barriers to use of prenatal services exist. He quoted studies indicating that removal of barriers will increase use of services. He explored reasons why access to care might be limited among poor patients in our society. To expand access to care, he called for fundamental social change to overcome existing barriers. Such changes might include improved transportation, child care, educational outreach, and improved insurance for the working poor.

His bottom line was most appropriate: "Only with a combination of medical and social effort can the infant mortality rate in America be reduced to a level that our citizens deserve and in which we can take pride."[22]

Perhaps Dr. Schwarz was responding to data from his own institution, King's County Hospital in Brooklyn, New York. From 133 patients delivered during 1981 to 1987, Delke et al.[23] found that maternal-social factors were associated more often than medical causes in avoidable perinatal deaths. In their study two thirds of the antepartum deaths and one third of the neonatal deaths were related to maternal-social causes.

They emphasized that social factors such as failure to utilize existing prenatal services are linked to low socioeconomic status, as are increased exposure to personal risk factors such as smoking and illicit drug use that are strong predictors of perinatal death.[23] Their study pointed out another important issue: Low socioeconomic status not only adversely influences perinatal mortality because of the high incidence of prematurity but also affects babies delivered at term or after term.

Dr. Schwarz's editorial raises a challenging question. Is the term "access to care," at least in some cases, misleading? Could this phrase divert attention away from a possibly more important basic problem such as underutilization of services? What are the factors that deny a patient access to care? The answers to these questions await comprehensive study. Likewise, why had the patient with eclampsia who died at Charity Hospital 32 years ago not presented for prenatal care?

The relation between smoking and low birth weight has been very well established.[24-27, 27A] A prospective study of 30,596 pregnant American women, controlled for maternal age, ethnicity, education, alcohol use, and eight other factors, indicated that preterm births were increased by 20% in women smoking one or more packs of cigarettes per day; the effect was strongest for births occurring before 33 weeks' gestation, where the excess was 60%.[28] Because of the large number of young

American women who smoke, cessation of smoking during pregnancy and reduction in the duration of prepregnancy smoking have a 39% potential to reduce the incidence of low-birth-weight babies.[11, 25]

A particularly onerous aspect of the tobacco industry has been its recent selective advertising in inner-city neighborhoods. Surveys in Baltimore, Detroit, Washington, St. Louis, and Atlanta have found that the vast majority of billboards in minority neighborhoods promote tobacco or alcohol.[29] Tinsley-Williams[30] criticized such billboards as "24-hour pushers."

In addition to targeting minorities, this industry has relentlessly and successfully projected the image of female smokers as glamorous and chic. Further, it portrays the young female smoker as energetic, sophisticated, and mature. The health consequences of cigarette smoking for the unborn have long been documented, and we now see that lung cancer has surpassed breast cancer as the leading cause of death from malignant neoplasia in women. The incidence and severity of benign chronic lung diseases as secondary consequences of cigarette smoking have soared.

Women are smoking more heavily, inhaling more deeply, and starting to smoke earlier than they did 20 years ago. Every day more than 1600 American teenage girls "light up" for the first time, and more girls are smoking than are boys.[31]

Surveys have demonstrated that women are more likely than men to use cigarettes as a means of coping with stress. Certainly this phenomenon has pertinence to inner city populations, probably to a greater degree than any other segment of our population.

Drug abuse, particularly abuse of crack cocaine, is an epidemic that burst on the nation in the 1980s. The effect on newborn babies has been severely deleterious, not only in neonatal mortality but in long-term morbidity in surviving infants.

Of 353 patients studied, Calhoun[32] found that 91 (25.8%) had positive tests for cocaine at the time of delivery. The prematurity rate was 37% in the cocaine-positive mothers versus 2% in cocaine-negative mothers. Two thirds of the infants had cocaine withdrawal.[32] Hunt[33] recently described a 700% increase in 4 years in drug-addicted neonates at Houston's Lyndon B. Johnson Hospital.

A poignant headline appeared in the *Louisville Courier-Journal* just 2 days ago. Under the title, "Hearing the Cries of Crack," Anna Quindlen[34] described the devastating effects of crack cocaine on neonates. Her statement in this article, "The chasm between rich and poor widens in utero," was certainly provocative. In response to this distressing situation, Dr. Mortimer Rosen[35] stated, "It's almost as if a new form of child abuse is arising in our world."

And, finally, acquired immunodeficiency syndrome, the other epidemic of the 1980s in America, is emerging as an infection with tragic consequences for mothers and babies that will have a growing impact on neonatal mortality and morbidity for the foreseeable future.

These problems are not isolated to inner-city America; they are also well recognized as contributing factors in perinatal mortality in Europe. They just happen to be more prevalent in America.

In every European country studied, the World Health Organization[36] found an increase in deaths with a decrease in socioeconomic status. It concluded that the interaction between socioeconomic status and biologic factors influences levels of perinatal mortality.

Dr. Marsden Wagner[37] recently stated the situation succinctly. On the basis of extended observations of health care delivery on both sides of the Atlantic, he states that, "Infant mortality is not a health problem. It is a social problem with health consequences." He emphasized the need for social support for pregnant women and infants. While I disagree with some of the recommendations he made in this article, his statement carries an important message.

Why hasn't this conclusion been emphasized in the popular press? Clearly, the challenge of reducing perinatal mortality in America is not restricted to changing the way medical care is delivered in our country. This is why it is hard to take the simplistic bashing of the medical profession that is aimed at dismantling our system of medical care.

Some intriguing data are available on medical care in Japan. Recall the major difference between Japan and the United States in neonatal mortality. They have been number one, while we have been number eighteen, mainly as an indication of the differences in social-socioeconomic status. Japan has much lower rates of drug abuse, teenage pregnancy, and babies born out of wedlock, and they have strong family support systems. In contrast to the low perinatal mortality in Japan, there is a substantial difference between the United States and Japan in **maternal** mortality rate, which is more a reflection of the adequacy of medical care. In 1975 the maternal mortality rates per 100,000 live births for Japan and the United States were 29 and 13, respectively.[38] This rate persisted in the 1980s—16 in Japan in 1985 versus 8 in the United States in 1983.[39] These figures indicate that medical care rendered to American mothers is significantly better than that care in Japan. Confirmation of this observation comes from Rosen and Fujimori,[40] who demonstrated that in England and Wales maternal mortality is significantly lower than in Japan; they discovered that the only difference among variables studied was availability of anesthesiologists. They speculated that this difference in medical care delivery was principally responsible for the higher maternal mortality in Japan. Thus the Japanese nation is doing better than our society from a social-behavioral standpoint as evidenced by neonatal mortality, but American medical care is better when maternal mortality is considered.

Revisiting the headlines presented at the outset, it is clear that some statements in the press are erroneous. ("In Japan, Canada, and many European countries, people live longer. . . ."[8]) In Japan, new mothers do not live longer.

Returning to another headline, I conclude, as did Representative Washington,[5] that our ***health care system,*** not necessarily our ***medical*** *care system,* must change; the major emphasis must relate to social-socioeconomic change.

What can we as a profession do to rectify this enormous problem of perinatal mortality? An exciting second example of leadership in this area exhibited by the American College of Obstetricians and Gynecologists was the recent presidential address by Dr. Ezra Davidson,[41] entitled, "A Strategy for Reducing Infant Mortality." In stressing that a woman who starts prenatal care in the first trimester has a fivefold better chance of having a living child than a woman who has no prenatal care, he made a clarion call for detailed, nationwide review of factors that are associated with every perinatal death in America. Because of the diversity of our society, the review will be conducted on a local basis, to include those factors unique to geography and local custom. It will focus not only on medical factors but on life-styles and living conditions, on access and utilization. It will hopefully address the relative importance and meaning of access versus underutilization. His words, "We must not medicalize our societal problems," beautifully emphasize the heart of one of my points today. It is incumbent upon all Fellows of the College, and all American physicians rendering perinatal care, to encourage and facilitate this study. For it is only when there is reasonable knowledge of the origin of the problem that appropriate steps can be taken to change the outcome. In addition, the data gained will most likely blunt or eliminate the attacks on so-called deficiencies in our medical care and emphasize to the American people the societal arena in which the principal challenges lie.

On the basis of the information presented today, I find it unlikely that a wholesale change in the medical care system in America will change our rank in perinatal mortality among nations. Instead, a societal change of major magnitude must be implemented, and the willingness of the American people to attack such basic problems must be put into action.

Perhaps some speculation is in order. Why do the northern Europeans and certain other countries have significantly lower perinatal mortality? It has long been postulated that the more or less homogeneous nature of their societies, which have relatively few truly poor people, permits a more uniform application of medical care.

Wallace et al.[20] in 1989 pointed out that Japan, Sweden, and Finland have shared the number one ranking for lowest infant mortality for a decade on a somewhat rotating basis.

The latter two countries have a widespread network of maternal health clinics that are very well attended by their patients. They report that 95% to 99% not only register for health care but register early. Women are monitored for high risk early and continuously. Both countries have an effective system of education of parents before and after delivery.[42] Thus they have a highly motivated, compliant population whose utilization of medical services occurs at a very high rate. Access to care is associated with a national health service supported by very high individual taxes. They have a lower percentage of teenage births, and sex education as a national policy is taught in primary and secondary schools in Sweden and Finland. Family planning education and services are freely available and are used extensively. Social benefits include allowances for all pregnant women, predelivery maternity leave with salary (60 days in Sweden; 30 days in Finland), and postpartum maternity leave with salary for 1 year.

Among other reasons for low infant mortality, the authors state that populations in Sweden and Finland are generally of high socioeconomic status and have a high standard of living, including education and nutrition. Also they have a broad system of social support. **Any** health care delivery system would shine under these circumstances.

The United States has been compared with other nations, none of which have populations as diverse as ours. None have opened their borders or their hospitals to refugees from around the world. I am not apologizing for our less than homogeneous society. In fact, our diverse population, our "melting pot," is associated with the finest system of government yet devised, and we are the culturally and technologically richest country in the world. We have arrived at this point, on the doorstep of the twenty-first century, because of a longstanding tradition of freedom of expression, freedom of choice, and freedom to work hard and reap the rewards of our labor. This has been the American way, and, as applied to medical care for a majority of Americans, freedom to choose a physician or a hospital and freedom for physicians to elect to treat patients has created the finest medical care system in the world.

In light of the material discussed herein, I feel that our medical care delivery system has not failed and is not second-rate because of our rank in perinatal mortality.

Nevertheless, one should not discount the damage done in moulding public opinion by constant media harping of the worst-case possibilities that exist in health care delivery. In addition to media's exaggeration of certain issues, failure to report the true nature of a health care issue diverts public attention away from the core disorder, as in the social origins of American perinatal mortality. Society's ability to deal with the problem is thereby compromised.

The media are not always negative in reporting medical affairs. On Oct. 3, 1990, Ms. Judy Woodruff on the PBS McNeill-Lehrer News Hour did a piece on infant mortality, which focused principally on the problem of low-birth-weight babies of inner city women, with strong emphasis on crack cocaine use during pregnancy as a cause of perinatal mortality and severe postnatal morbidity. Rather than attacking medical care, the focus was on low-birth-weight babies in the General Hospital Neonatal Intensive Care Unit in Washington, D.C., and the reasons why they were there. The image of those suffering babies presented a very powerful message; the media in working together with the medical profession could expand this type of reporting to tremendously benefit American mothers and babies.

Herein lies a hope of spring.

The role of the media in America has become increasingly more important, because the huge potential for disseminating information can shape the actions and habits of people in all walks of life. In no other health care area could a shift from a negative to a positive message be of more importance than in infant mortality.

In fact, an aggressive, positive, hopeful television campaign could be the linchpin of a program to heighten public awareness and stimulate patients to utilize available prenatal and postnatal services or to learn where and how to get the help they and their babies so desperately need. Television may or may not be the best way; in some cases it **might be the only way** for the health care message to reach deep inner-city or distant rural areas of America.

In reflecting on how potent the media have been in projecting the poor image of American medicine, I realized that they might be equally powerful in projecting a positive health care message related to infant mortality. With the proper format varied to appeal to diverse target audiences, with the use of role models appropriate for those targets, with input from concerned obstetricians and pediatricians, as well as other health care providers, and with adequate funding, a compelling message repetitively broadcast would likely save more infants' lives than would any other single societal effort.

With confidence that such an aggressive program has a chance to succeed, I truly feel **the hope of spring.**

I also feel confident in saying that there is not a major reason to radically change our medical care system, certainly not because we have failed in the area of perinatal mortality. It is ironic to see the escalating calls for centralized, nationalized, or socialized medicine at a time in modern history when many socialistic nations are crumbling and are speeding to rearrange their societies toward market principles that are based on freedom of choice and individual productivity, perhaps emulating the American way.

What tradition brought American medicine to preeminence in the world? What must we protect to ensure optimum medical care for neonates and most Americans in the next century? What must we *not* change?

We must not change the doctor-patient relationship that is based on the traditional private practice model. This is the best model for medical care delivery in the United States. Surveys have repeatedly documented that whereas people may be disgruntled with some aspects of health care, they remain consistently satisfied with their private physician.

American patients want freedom of choice, and autonomy, and privacy. They want to have their own physician who can be understanding, caring, and expert. And they want one who will be their friend.

In England, where once there was near-total government medicine, there are now private hospitals, private practice, and private insurance serving about 10% of the population; since 1981 the growth has been steady at 5% per year. In 1983 23% of professionals and 22% of employers and managers had private medical insurance. In spite of his support for the National Health Service, Vayda[13] states there is a general level of anxiety about the ability of the NHS to meet health care needs, which reinforces the appeal of private alternatives.

It is likely that a pluralistic system will evolve in America. There will be a continued responsibility of local, regional, and federal governments to provide health care for those who **cannot,** or prefer not to, seek private medical care. Also the federal government will continue to be involved with the Veterans Administration system, Public Health Service, Armed Services, etc. A logical area of extension of the federal government would be to provide insurance for all pregnant women and their infants who cannot provide their own means. This would eliminate the lack of prenatal care for purely financial reasons. Would it not be wonderful if such a system permitted establishing a physician-patient relationship for our clinic patients, without major intrusion by the government? You know what that would do for utilization.

For many poor Americans without hope, such a program might also be a **hope of spring.**

Many of us, and likely a majority of the membership of this association, will continue to practice one-on-one medicine on patients who have sought us out, will pay us, and whom we will willingly and proudly serve during the remainder of our professional lifetimes. They will come to us because we have developed an individual style or an area of expertise, because we are caring and competent physicians who are willing and happy to work long hours to serve their needs.

There will be ever-growing numbers of patients who will be involved with one plan or another who will seek health care in a less personalized setting delivered by physicians who will have less interest in the patient as a person and who will function more in the 9-to-5 mode.

But there will remain a substantial number of American patients who will pursue a private doctor-patient relationship, who will remain friends with their physician, and who will solve their health care problems in the American way—they paid for it because they worked for it, because they earned it.

In spite of all the depressing rhetoric, the criticism I call the "bleak winter of our profession," there are many physicians whose voices have been unheard. These are people who have not been interviewed; thus they are not in the statistics. Included among these are many who do not complain about how life, circumstances, and bureaucrats have wronged them. They do not complain because they are too busy—busy carrying out the day-to-day problem-solving and challenges of the practice of medicine, with its usual triumphs, occasional heartbreaks, but always fascinating interactions with patients, colleagues, and allied health professionals.

From my viewpoint, I still have to "pinch myself" to realize that I have been living a lifelong dream to be a physician—and I find it hard to believe that I get paid well to do work that gives me pleasure and satisfaction.

I still see young physicians and students who have that attitude. They are committed students who will one day be fine physicians. They are willing to hang onto a dream; whereas it was easy for you and me to pursue our dream, these students are bombarded with negativity regarding their dream. Perhaps those who persist and establish that precious patient-physician relationship will cherish what they have earned even more than we have.

Therein lies the **hope of spring**—the continuation of this attitude into the next century, so that the practice of medicine in America will remain strong and continue to attract bright individuals on whom the health of this nation will depend.

This **hope of spring** is also embodied in those of us who have learned and practiced and taught in the greatest system of health care delivery this planet has ever known. Continuation of the heart of this system, the private practice of medicine, is dependent on our hard, honest work, our example, and our counsel to the young people around us.

I wish to thank Mrs. Billie Ann Uralinak for her assistance in the preparation of this address.

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The mammary stimulation test—A predictor of preterm delivery?

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Clinical risk identification for preterm delivery, the major cause of perinatal morbidity and mortality, remains problematic. To test the hypothesis that a provocative test for uterine contractility at the beginning of the third trimester would reliably identify patients destined to be delivered before and at term, we designed the mammary stimulation test. Uterine contractions in response to nipple stimulation at a mean of 28.6 weeks' gestation, were evaluated in 94 gravid patients at risk for preterm delivery. The mammary stimulation test, the results of which were not used for clinical management, was positive in 50% of patients tested and had a sensitivity of 84% and a positive predictive value of 34% ($\chi^2 = 11.15$, $p < 0.01$). Ninety-four percent of patients predicted to be delivered at term actually were delivered at term (negative predictive value). Furthermore, no patients with a negative mammary stimulation test were delivered within 1 month of testing or were delivered of infants weighing <2000 gm. Discriminant analysis indicated that the mammary stimulation test provided information beyond that available from clinical risk factors alone. A cost analysis suggested that the use of the mammary stimulation test could reduce the cost of ambulatory uterine activity monitoring by nearly 50%. If these findings can be validated in additional samples, the mammary stimulation test may be useful in prematurity prevention programs. (AM J OBSTET GYNECOL 1991;164:1409-19.)

Key words: Mammary stimulation test, prematurity, nipple stimulation

Preterm birth remains the major cause of perinatal morbidity and mortality and results in significant expenditures of health care resources.[1,2] Preventing prematurity is highly desirable but is hampered by the limitations of clinical risk assessment. Current programs, including clinical risk assessment, patient behavioral modification, ambulatory uterine activity monitoring, and tocolysis, have not produced a decrease in the incidence of prematurity.[1,3,4]

Clinical risk assessment and scoring for preterm delivery are based primarily on epidemiologic and medical factors, including selected social and demographic factors, obstetric and medical histories, and current pregnancy complications.[5-7] Although risk scoring systems are helpful in identifying women destined to be delivered prematurely, they have low sensitivities and positive predictive values.[6] The addition of ambulatory uterine activity monitoring may contribute to improved identification of the at-risk patient,[8-11] but its cost-effectiveness is questionable.[4,12-16]

Our hypothesis, which is based on a theoretical consideration of the endocrine control of parturition, states that a provocative test of uterine contractility at the beginning of the third trimester could identify patients destined to be delivered prematurely. We designed the mammary stimulation test for this purpose. This study prospectively evaluates the ability of the mammary stimulation test to predict preterm delivery in a sample of at-risk patients.

Methods

During a 6-month period, 94 gravid patients who were between 24 and 32 weeks' gestation and were attending a core-city antenatal clinic for the medically indigent were enrolled in a research protocol approved by the Human Investigation Committee of Wayne State University. Demographic factors, gestational duration, and clinical risk factors were recorded. After written informed consent was obtained, the patient was placed in semi-fowler's position. Doppler fetal heart rate was obtained, and a tokotransducer was applied (Hewlett-

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6/6/28940

Table I. Clinical characteristics, antenatal risk factors, and mammary stimulation test results for preterm and term delivery groups

	Preterm	*Term*	*Total*
No. of patients	19 (20%)	75 (80%)	94 (100%)
Maternal characteristics			
Maternal age (yr)	23.7 ± 6.5	23.1 ± 5.3	23.2 ± 5.5
Gravidity	3.5 ± 2.1	3.2 ± 2.1	3.2 ± 2.1
Parity	1.3 ± 1.1	1.2 ± 1.4	1.2 ± 1.3
Gestational duration at testing (wk)	28.9 ± 2.2	28.5 ± 2.2	28.6 ± 2.2
Antenatal risk factors			
Major factors	13 (68%)	24 (32%)	37 (39%)†
Previous preterm delivery*	9 (47%)	12 (16%)	21 (22%)†
Multiple gestation*	3 (16%)	2 (3%)	5 (5%)†
Cervical incompetence*	2 (11%)	3 (4%)	5 (5%)
>1 previous*	5 (26%)	9 (12%)	14 (15%)
Cone biopsy	0 (0%)	3 (4%)	3 (3%)
Minor factors			
1 Previous elective abortion	3 (16%)	18 (24%)	21 (22%)
Late prenatal care*	4 (21%)	4 (5%)	8 (9%)†
Maternal stress	2 (11%)	6 (8%)	8 (9%)
Pregnancy <12 mo	3 (16%)	3 (4%)	6 (6%)
Fibroid uterus	2 (11%)	3 (4%)	5 (5%)
Chronic urinary tract infection	0 (0%)	4 (5%)	4 (4%)
Maternal age <16 yr	1 (5%)	2 (3%)	3 (3%)
Bleeding after 18 wk	0 (0%)	2 (3%)	2 (2%)
Positive mammary stimulation test	16 (84%)	31 (41%)	47 (50%)†

*Risk factors used in discriminant analysis (Table V).
†$p < 0.05$.

Packard 8040A). According to a standard protocol of nipple stimulation contraction stress testing,[17] baseline uterine activity was evaluated for at least 10 minutes, after which the patient lightly stimulated one breast (nipple) through her clothing for 2 minutes. Stimulation was discontinued if contractions occurred. If contractions did not occur, the same protocol was repeated after a 2-minute rest period for up to three cycles. If there was still no uterine activity, the patient first performed unilateral stimulation for 10 minutes, followed, if necessary, by bilateral stimulation for another 10 minutes. When uterine activity subsided, we discontinued monitoring.

All patients had management by resident and staff physicians who did not know the mammary stimulation test results. The primary outcome measure, which was chosen a priori, was preterm delivery, which we defined as delivery before 37 completed weeks' gestation by best obstetric estimate. Secondary outcome measures included preterm labor, the need for tocolysis, and test-to-delivery interval.

Contingency tables were constructed to evaluate the relationship of mammary stimulation test results to outcome measures. In exploratory analyses, differences in continuous variables were evaluated by the Student t test, and categoric variables were evaluated by χ^2. We used discriminant analyses, which included the independent variables of candidate clinical risks and mammary stimulation test results and the dependent variables of preterm delivery and preterm labor, to determine whether mammary stimulation test results provided information beyond that available from clinical risks. A p value of <0.05 was considered significant.

Results

Of the 94 patients in the study sample, 19 (20%) had preterm delivery. Clinical characteristics, antepartum risks, and mammary stimulation test results (independent variables) are shown in Table I. As we anticipated, preterm delivery was preceded by an excess of recognized antepartum medical and obstetric risk factors. The preterm and term delivery groups did not differ in the gestational duration (approximately 28.5 weeks) at which the mammary stimulation test was performed. However, the mammary stimulation test was positive about twice as frequently among the patients who had preterm delivery than among those who were delivered at term (84% vs 41%, respectively). Table II documents pregnancy outcomes, including the primary and secondary outcome measures (dependent variables). Among the 19 patients who were delivered before term, only one infant died in the neonatal period. There were no complications attributable to mammary stimulation test testing.

Table II. Outcome measures for preterm and term delivery groups

	Preterm	*Term*	*Total*
No. of patients	19 (20%)	75 (80%)	94 (100%)
Preterm labor	6 (32%)	10 (13%)	16 (17%)
Tocolysis	4 (21%)	8 (11%)	12 (13%)
Mammary stimulation test–Delivery interval (wk)	4.3 ± 2.3	10.7 ± 2.8	9.1 ± 3.8*
Gestational duration (wk)	33.5 ± 2.2	39.7 ± 1.1	38.2 ± 3.0*
Birth weight (gm)	1987 ± 431	3291 ± 573	2961 ± 784
Cesarean section	6 (31%)	7 (9%)	13 (14%)*
Required NICU stay	10 (53%)	3 (4%)	13 (14%)*
Maternal postpartum stay (days)	3.0 ± 1.5	2.7 ± 1.5	2.8 ± 1.5

NICU, Neonatal intensive care unit.
*$p < 0.05$.

Table IIIA. Relationship of mammary stimulation test results to preterm delivery

Mammary stimulation test	*Preterm delivery*		*Total*
	Yes	*No*	
Positive	16	31	47
Negative	3	44	47
TOTAL	19	75	94
χ^2	11.15		
p Value	<0.01		
Odds ratio	7.6 ± 5.1		
Relative risk	2.0		
Sensitivity	84%		
Specificity	59%		
Positive predictive value	34%		
Negative predictive value	94%		
At risk	50%		
Explained variance	12%		

Table IIIB. Relationship mammary stimulation test results to preterm labor

Mammary stimulation test	*Preterm labor*		*Total*
	Yes	*No*	
Positive	23	24	47
Negative	8	39	47
TOTAL	31	63	94
χ^2	10.83		
P value	< 0.01		
Odds ratio	4.7 ± 2.3		
Relative risk	1.9		
Sensitivity	74%		
Specificity	62%		
Positive predictive value	50%		
Negative predictive value	83%		
At risk	50%		
Explained variance	12%		

Relationships of mammary stimulation test results to the primary and secondary outcome measures are shown in the contingency tables (Tables IIIA, IIIB, and IIIC). The odds that a patient with a positive mammary stimulation test would be delivered before term were increased 7.6-fold. A positive mammary stimulation test correctly identified 16 of the 19 patients who were delivered before term (sensitivity = 84%). In addition, a negative test preceded term delivery in 44 of 47 patients (negative predictive value = 94%). Among the three patients with negative mammary stimulation tests who were delivered before term, delivery occurred after 34 weeks' gestation, none had a test-to-delivery interval of <5 weeks. Furthermore, all babies born to mothers with negative mammary stimulation tests weighed >2000 gm.

Mammary stimulation test results were related to the occurrence of preterm labor with a strength similar to that for preterm delivery (explained variance = 12%). No patient with a negative mammary stimulation test was delivered during the month after the test (positive predictive value = 100%), and all patients who were delivered during the succeeding month had a positive mammary stimulation test (specificity = 100%).

To evaluate the potential role of the mammary stimulation test in clinical care, test results were related to preterm delivery in patients with the major risk factors shown in Table I. The results of this analysis are shown in Table IV. Limiting use of the mammary stimulation test to high-risk patients doubles the explained variance (24%) of the classification. However, comparing Tables IIIA, IIIB, IIIC, and IV reveals that six patients who were delivered before term did not have major high-risk factors and that five of these had positive mammary stimulation tests.

Finally, to better understand the relationship of mammary stimulation test results and clinical factors as potential predictors of preterm outcomes, we per-

Table IIIC. Mammary stimulation test–to–delivery interval

Mammary stimulation test	*Mammary stimulation test–to–delivery interval*		*Total*
	≥5 wk	*<5 wk*	
Negative	47	0	47
Positive	35	12	47
TOTAL	82	12	94
χ^2	13.76		
p Value	<0.01		
Relative risk	2.3		
Sensitivity	57%		
Specificity	100%		
Positive predictive value	100%		
Negative predictive value	26%		
At risk	50.0%		
Explained variance	15%		

Table IV. Relationship of mammary stimulation test results to preterm delivery in high-risk patients

Mammary stimulation test	*Preterm delivery*		*Total*
	Yes	*No*	
Positive	11	8	19
Negative	2	16	18
TOTAL	13	24	37
χ^2	8.88		
p Value	<0.01		
Odds ratio	11.0 ± 9.7		
Relative risk	2.5		
Sensitivity	85%		
Specificity	67%		
Positive predictive value	58%		
Negative predictive value	89%		
At risk	51%		
Explained variance	24%		

formed two discriminant analyses, that allowed the clinical factors to be entered before the mammary stimulation test results. In addition to demographic data, clinical factors included the risk factors indicated by asterisks in Table I. Two antenatal risk factors, previous preterm delivery and late prenatal care, contributed significantly to the classification of preterm delivery [F (2,91) = 8.9, R^2 = 16.4%]. The mammary stimulation test significantly improved the classification by adding an additional 7.7% of explained variance [F (3,90) = 9.5, R^2 = 24.1%]. Four clinical factors contributed significantly to the classification of preterm labor [F (4,89) = 12.4, R^2 = 35.8%]. The mammary stimulation test significantly improved this classification, as well, by adding an additional 5.2% of explained variance [F (5,88) = 12.3, R^2 = 35.8%]. The resulting jackknifed classifications are shown in Tables VA and VB.

Comparison of Table VA with Table IIIA reveals that, although including clinical risks doubles the explained variance and improves the specificity from 59% to 91%, six cases of preterm birth, which are correctly classified by the mammary stimulation test alone, are misclassified when clinical risks are taken into account, thereby decreasing the sensitivity from 84% to 53%. Comparing Table IIIB with Table VB reveals that considering clinical risks improves the positive predictive value from 49% to 73% and the specificity from 62% to 87% without substantially diluting the sensitivity.

Comment

The key finding in this study is that a new provocative test, the mammary stimulation test, predicts preterm delivery and can be used to predict both preterm labor and not being delivered within 1 month of testing. In addition, the mammary stimulation test provides more extensive information than do the usual clinical risk factors considered useful for identifying patients destined to deliver before term.

The seed that led to our development of the mammary stimulation test derived from exposure of one of the authors (R.J.S.) to a technique used by Drs. Arpad Csapo and Jacques Sauvage at Washington University in St. Louis more than two decades ago. Uterine response to oxytocin challenge was used as a marker of the evolution of uterine contractility[18] to predict whether induction of labor would be successful and to

Table VA. Jackknifed classifications from discriminant analysis for preterm delivery

Mammary stimulation test	*Preterm delivery*		*Total*
	Yes	*No*	
Positive risk	10	7	17
Negative risk	9	68	77
TOTAL	19	75	94
Odds ratio	10.8 ± 6.6		
Relative risk	5.0		
Sensitivity	53%		
Specificity	91%		
Positive predictive value	59%		
Negative predictive value	88%		
At risk	18%		
Explained variance	24%		

Risk factors: MST results, previous preterm delivery, late prenatal care.

Table VB. Jackknifed classifications from discriminant analysis for preterm labor

Mammary stimulation test	*Preterm labor*		*Total*
	Yes	*No*	
Positive risk	22	8	30
Negative risk	9	55	64
TOTAL	31	63	94
Odds ratio	16.8 ± 8.2		
Relative risk	5.2		
Sensitivity	71%		
Specificity	87%		
Positive predictive value	73%		
Negative predictive value	86%		
At risk	32%		
Explained variance	12%		

Risk factors include: mammary stimulation test results, gravidity, abortions, multiple gestation, and previous preterm delivery.

answer the question, "Is the myometrium in a state that is ready for labor?"

During the past two decades, substantial research has been used to explain the basis for the evolving uterine response to oxytocin challenge.[19] It appears that uterine sensitivity to oxytocin increases with advancing gestational age as a result of an increase in the number and effectiveness of myometrial oxytocin receptors.[20-22] Because oxytocin sensitivity increases before the onset of labor, several investigators have correlated uterine activity, oxytocin sensitivity, and perterm labor and delivery.[19] Uterine activity and oxytocin sensitivity are increased in patients who are delivered before term.[23-26] Finally, a separate line of research indicates that nipple stimulation results in oxytocin release and elevation of plasma levels of oxytocin.[27-29] As a result of these studies, we speculate that in patients destined to be delivered before term, the mammary stimulation test reveals the state of the myometrium as marked by increased sensitivity to oxytocin endogenously released by nipple stimulation.

The results presented here should be interpreted cautiously. This initial series of patients is small. Validation in larger samples with different demographic characteristics will be necessary. In particular, the utility of the mammary stimulation test must be tested in samples with lower prevalences of preterm labor and delivery. An additional caveat is that the initial criterion for mammary stimulation test positivity was simply stated in the study protocol as "the presence of uterine contractility." More specific criteria were derived heuristically by one of the investigators (R.D.E.) in conjunction with interpreting the tracings. The resulting criteria are shown in Table VI. To be classified as a positive mammary stimulation test, uterine activity (contractions >40 seconds in duration) should be noted before the onset of stimulation (Fig. 1) or in response to nipple stimulation before the end of the second 2-minute rest period (Fig. 2). To be classified as a negative mammary stimulation test (Fig. 3), uterine activity should not be observed before the end of the second rest period (8 minutes after the onset of unilateral breast stimulation). In this series all mammary stimulation tests that became positive did so before 8 minutes after the onset of unilateral stimulation. Although interpretation of the tracings was entirely blinded as to clinical outcome, these recommended criteria also require validation.

Table VI. Mammary stimulation test criteria

1. Positive test
   a. Any spontaneous uterine contraction(s)>40 seconds before initiation of nipple stimulation
   b. Any uterine contraction(s) of >40 seconds by completion of two stimulation cycles (8 minutes from the onset of stimulation)
2. Negative test
   Absence of uterine activity [contraction(s) that last for >40 seconds before or during stimulation.

Despite the caveats noted in the previous paragraph, the mammary stimulation test appears to have considerable promise for clinical application. It appears to be more sensitive in detecting risk for prematurity than

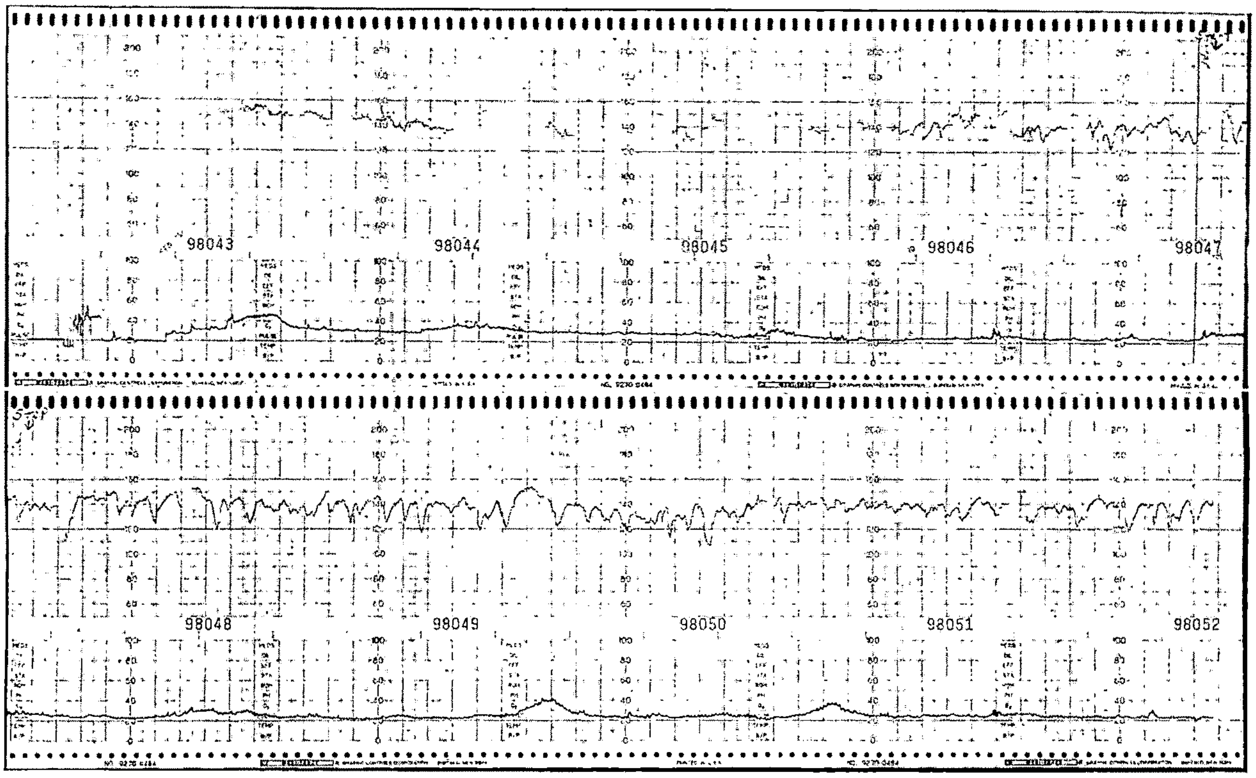

Fig. 1. Spontaneous positive mammary stimulation test. Spontaneous uterine contraction(s) are noted before initiation of nipple stimulation.

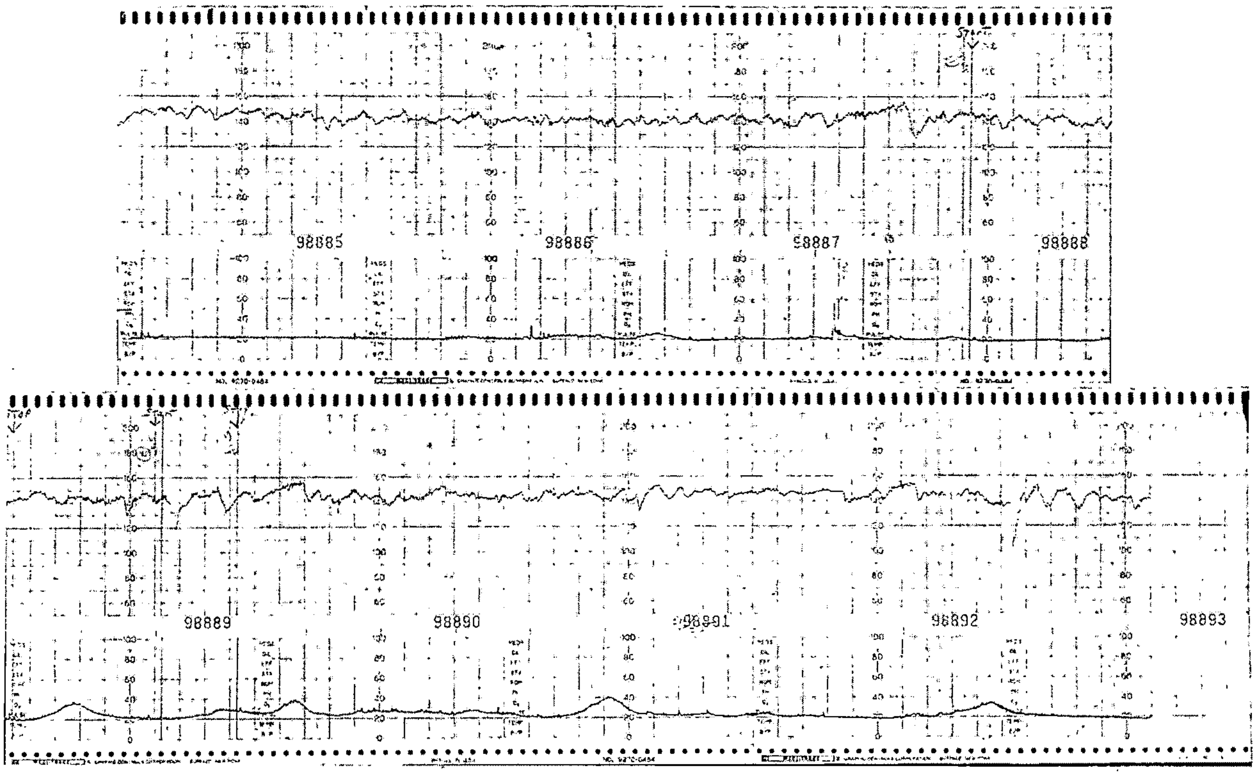

Fig. 2. Positive mammary stimulation test. Uterine contractions are noted by completion of two stimulation cycles.

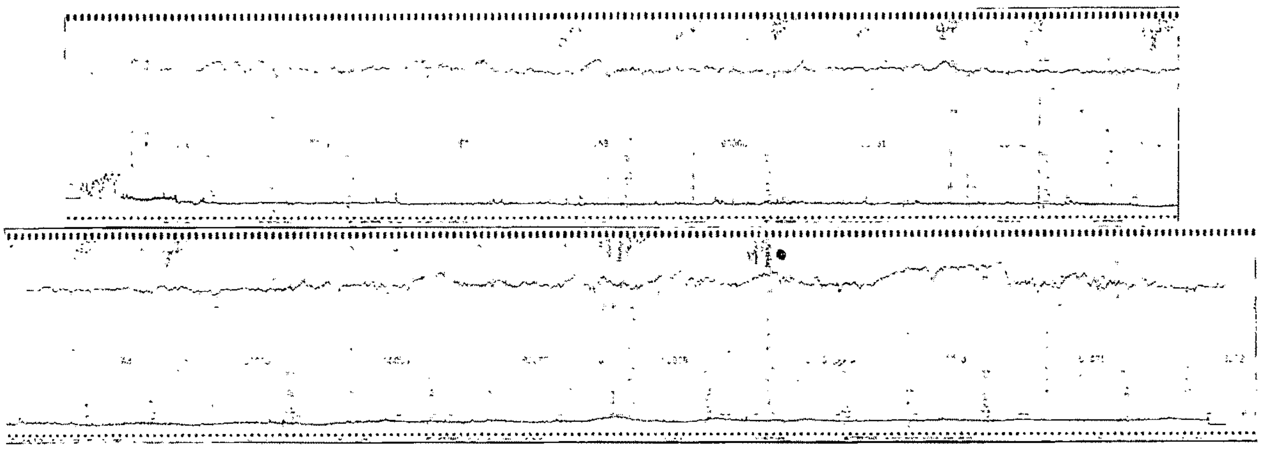

Fig. 3. Negative mammary stimulation test. Absence of uterine activity before or during stimulation.

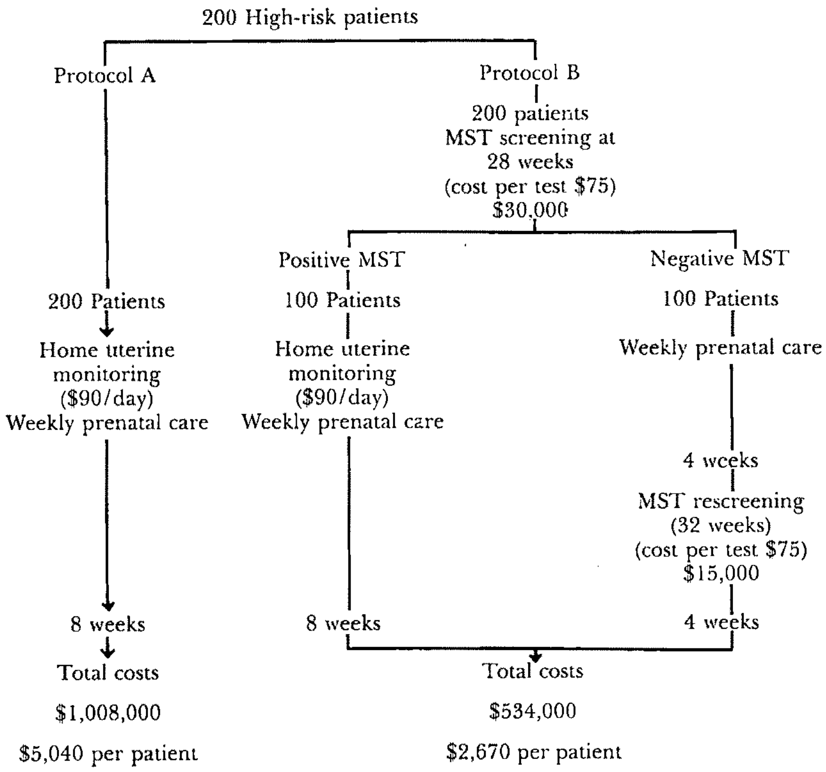

Fig. 4. Potential cost savings of home uterine activity monitoring when preceded by mammary stimulation test *(MST)* screening.

are clinical factors. The nipple stimulation and monitoring methods[17] are well validated and safe and currently are widely used in clinical practice to perform contraction stress tests. Performing the mammary stimulation test usually requires about 20 minutes, which is a duration between that needed for fetal nonstress testing and that for contraction stress testing. In addition, the mammary stimulation test appears to reliably identify patients who will *not* be delivered prematurely. Thus it appears that mammary stimulation test testing could be a highly cost-effective component of prematurity prevention.

Currently, formal prematurity prevention programs consist of clinical risk assessment, patient behavioral modification, and ambulatory uterine activity monitoring. The most controversial and the most expensive of these techniques is ambulatory uterine activity monitoring, and its contribution to prematurity prevention is controversial. With a method such as the mammary stimulation test, which is more accurate than clinical risk assessment in predicting preterm delivery, the numbers of patients requiring daily uterine activity monitoring might be reduced, and cost-effectiveness might be improved.

Fig. 4 depicts a cost analysis of an ambulatory uterine activity monitoring program both with and without mammary stimulation test screening, it uses assumptions that are based on observed rates in the current series. Monitoring is routinely begun at 28 weeks' gestation and continues for 8 weeks in a hypothetical sample of 200 patients at risk for preterm delivery. Under protocol A, all patients are monitored at home at a cost of $90 per day, which results in a total cost in excess of $1 million. However, if mammary stimulation test screening is used at 28 weeks at a cost of $75 per test to determine risk status, 100 patients (those with negative mammary stimulation tests) do not require home uterine activity monitoring (protocol B). Because the predictive value of a negative mammary stimulation test persists for at least 5 weeks, patients are retested in 1 month (32 weeks) to reevaluate risk status. The net savings of the mammary stimulation test screening program are nearly half the cost of an ambulatory uterine activity monitoring program.

In conclusion, the mammary stimulation test appears to have the theoretical rationale, predictive utility, simplicity, safety, and low cost that recommend additional study to determine whether it should play a major role in the care of pregnant women.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Washington C. Hill, Nashville, Tennessee. Predicting which patients will develop preterm labor or will be delivered before term is, at this time, fair at best. Eden and co-workers have joined the ranks of many of us in trying to devise a method to predict which myometrium is susceptible to going into labor before term. They do this by designing a new test with new initials, the MST, thereby adding another eponym of obstetrics (EOO) to an already crowded perinatal alphabet soup (FCI, L/S, AST, CST, OCT, BSST, D/A, PTL, NST, AFV, FAD, PUBS, FSI, A/B, S/D, FBP, etc.). This test utilizes the breast stimulation stress test in attempting to predict which patients may develop preterm labor or have preterm delivery.

It will be difficult at best for any one test, clinical evaluation, or system to predict preterm labor or preterm delivery. The individual methods alone are simply not sensitive enough or do not have a high enough positive predictive value. A combination of these methods in forming a preterm labor screening index will probably be more successful.

I would like to critique this article by looking at the five characteristics, mentioned by the authors, that make [this topic] worthy of further study: theoretical rationale, predictive utility, simplicity, safety, and low cost.

The theoretical rationale of the mammary stimulation test is based on a difference in the myometrium that will go into labor early versus the myometrium that will progress into labor at term. It is theorized that there are more numerous or more sensitive oxytocin receptors in the former. Data suggest that this may indeed be the case. There is, however, a difference between (a) using known amounts of exogenous oxytocin to see if the uterus is ready for labor and (b) using unknown amounts of endogenous oxytocin produced by nipple stimulation to see if preterm labor will develop or preterm delivery will occur. Point a is not equal to point b.

Eenie, meenie, mini, mo: What do the data really show about predictive utility? The reader is bombarded with many statistics: positive predictive value, sensitivity, specificity, negative predictive value, percent at risk, χ^2, explained variance, relative risk, odds ratio, p value, jackknifed classifications, discriminant analysis, and contingency table. The "bottom line" appears to be that with a small number of patients the sensitivity, specificity, positive predictive value, and negative predictive value of the mammary stimulation test are in the same range as those of other reported methods devised for predicting preterm labor or preterm delivery. In fact, in assessing the predictability of the mammary stimulation test, risk scoring is also used. The ability to predict preterm delivery or term delivery is also affected and confused by introducing and confounding variable of tocolytic therapy. Some patients who had a positive mammary stimulation test and subsequently were delivered before term or at term had tocolytic therapy. The same is true for some who had preterm labor.

The predictive value of a negative mammary stimulation test for preterm delivery was very high in this small number of patients, but it certainly was not reliable enough in this preliminary report to eliminate home uterine activity monitoring from a prematurity prevention program because three patients were delivered preterm. The real negative predictive value has yet to be determined.

The mammary stimulation test is simple and easy to perform. As with the breast stimulation stress test, there will be patients who prefer not to stimulate themselves. Although the test is simple, will it be useful or predictive?

I don't know if the mammary stimulation test is safe; it may be, but there are no data on the interval from the administration of the mammary stimulation test to the onset of preterm labor. Could the mammary stimulation test (performed correctly or incorrectly) cause preterm labor, preterm delivery, or both in higher-risk patients? Which came first, the chicken or the egg? Could performing the mammary stimulation test cause hyperstimulation? As the authors mentioned, it is necessary to study more patients with variable risks to answer these and other important questions.

The test is inexpensive, but this is only one variable in determining its real value. An inexpensive useless test is still a useless, cheap test.

I agree with the authors that the final verdict on the mammary stimulation test is not in yet and that we should be cautious in interpreting this limited experience. I have the following questions for them, whom I commend for winning the Annual Prize Award and for making an excellent attempt in tackling a very difficult problem that is elusive to many investigators.

1. The authors note that *no* patients with a negative mammary stimulation test were delivered before 34 weeks or had a baby weighing <2000 gm. What was the similar breakdown for patients with a positive mammary stimulation test?
2. What was the interval from the mammary stimulation test to the onset of preterm labor?
3. What was the regimen of tocolysis, and how do the authors deal with this confounding variable when interpreting their data?
4. How was preterm labor defined?
5. All studies reporting on the breast stimulation stress test have involved hyperstimulation. What was the incidence in this study?
6. How early could this test be used?

Dr. Randall T. Kelly, Dearborn, Michigan. I do not feel that this article will cause a revolution in perinatal medicine, but I do feel that the practice of obstetrics will change in the future as a result of the authors' pioneering work on uterine contraction activity testing. The article deserves little criticism, but there are a few things that I would like to discuss.

If a test in medicine is to be universally performed, as I feel that this one (or something similar to it) will be on all pregnant patients, then nomenclature that is more easily accepted and understood by physician and layman alike will help us to maintain that test in the public domain. In addition, pregnant patients will be more willing to accept it as a routine test of pregnancy. Perhaps a better name for the test would be the breast stimulation test for preterm labor or the nipple stimulation test for preterm labor. In any case, removal of the word *mammary* would be helpful, because many of our patients do not know what that word means. I think that there should be something about preterm labor in the name of the test.

In the first paragraph of the article, Dr. Eden states that with all of our current programs, there has been no decrease in the incidence of prematurity. This is true. The problem is, though, that many patients have no access to any program, no discussions about preterm labor and its symptoms are provided during prenatal care.

In the Methods section, a reference is listed for the standard protocol of nipple stimulation contraction stress testing. This reference, which is from Dr. Eden and Dr. Boehm's textbook, does not have a protocol for the nipple stimulation contraction stress test in that chapter as listed in the reference.

There is a big range of gestational ages in this study (24 to 32 weeks). One could say that sensitivity to oxytocin or other hormones could differ significantly at 24 weeks when compared with 32 weeks, and so using fetuses of one gestational age would make a "cleaner" study.

Exactly how the nipple is stimulated is not discussed, nor is whether the stimulation is continuous for the 2-minute period. Stimulation was discontinued if contractions occurred. Later, it appeared that even one contraction was sufficient for discontinuation. There should be a discussion of criteria regarding these contractions, and what is considered a positive or a negative mammary stimulation test should be established.

I also have a few questions. In Table I, did the patients with cervical incompetence have a cerclage? This should be delineated. In Table II only 6 of the 19 patients who were delivered before term had preterm labor. Were some patients delivered early because of a medical problem, such as severe preeclampsia, which might make the uterus more sensitive to oxytocin at that stage of pregnancy? This should be discussed.

There is a statement that nipple stimulation results in oxytocin release and in an increase of plasma levels of oxytocin. This is still a slightly controversial point that could have been examined with plasma oxytocin levels in this study. Perhaps a plasma oxytocin level, prostaglandin level, or prostaglandin metabolite level at a certain stage in pregnancy will be our future test with better sensitivity.

The overall series is small. The authors realize this. The participants in the study were not randomly selected; this limits the ability to generalize the findings. The study considers a group of patients who are at an extremely high risk for preterm labor. Another study on groups with a much lower risk for preterm labor (and thus perhaps with fewer confounding variables, such as poor socioeconomic status, previous preterm delivery, and late prenatal care) would make a much "cleaner" study. Again, the criteria for positive and negative tests are not dealt with until the Comment section and Table VI. Even though the criteria for contractions and positive and negative testing were originated heuristically, I feel that they should have been discussed in the Methods section so that the reader would understand the remainder of the article without having to read it many times.

The statistical analyses are complete and appropriate for the type of data collected and for the nature of the research project, but the authors often did not explain or discuss the statistics or the analyses sufficiently. More description and interpretation of the tables would have been useful and would have made the data more understandable to the reader. Very specific data are provided with minimal comments in the tables.

The authors do state that the results of the study should be interpreted cautiously. I agree with this. However, I do feel that this is a good solid pilot study. In the future a test similar to the mammary stimulation test, as described here, or some other test along the same lines will be routine for all obstetric patients.

DR. ROBERT J. CARPENTER, Houston, Texas. One thing I didn't see was a breakdown of singleton versus twin gestations and their data base. I would like to have some information on that and on how the removal of twins may change the data.

DR. WILLIAM J. OTT, St. Louis, Missouri. I would like to congratulate the authors on their excellent pilot study and in trying to predict those patients at risk with premature labor.

I have one question, however, about the statistical analysis of their data. The positive and negative predictive values of any test are significantly influenced by the incidence of the disease process in question. I want to know whether Bayesian analysis was used to correct for this in determining positive and negative predictive values, because this may significantly alter the values of the positive and negative tests.

DR. SUZANNE R. TRUPIN, Champaign, Illinois. Were cervical examinations done on these patients? If so, were there any criteria for eliminating patients from the study or for determining a difference in responsiveness to the mammary stimulation test in patients with cervical effacement? Also, was there a difference between the multiparous patient and the nulligravid patient in response to this test?

UNIDENTIFIED SPEAKER, St. Louis, Missouri. I enjoyed the presentation. It is refreshing to have the results of some form of testing for preterm labor that will not increase (and may decrease) the use of home uterine activity monitoring.

I have two questions. One has to do with the incidence of preterm delivery in the group of patients from the same clinic who did not undergo any mammary stimulation testing. What is the baseline in the true control group?

Second, preterm labor really results from a hodgepodge of different causes, and I would like to have a breakdown, if possible, regarding patients who had placental complications with bleeding manifestations in preterm labor, membrane rupture in preterm labor, and also intact membranes with preterm labor, because there may be different abilities to predict these different causes of the actual birth event.

Dr. Eden (Closing). We like to thank the reviewers for their comments, especially that regarding alphabet soup.

Because we believe that the mammary stimulation test will, in fact, prove to be clinically useful, we agree that it is imperative that the test be given a simple, catchy, and easy-to-remember name. Our biggest dilemma was, in fact, deciding on the name of this provocative new test.

We also agree with the concerns raised regarding the predictive ability of the mammary stimulation test, and we are currently expanding the series considerably. Our fondest hope is that other institutions and investigators will attempt to verify our findings, and thus will enable the mammary stimulation test to take its place in prematurity prevention programs.

Three minutes will not permit me enough time to comment on all the questions, but I would like to address a couple of Dr. Hill's queries first.

We analyzed the patients who were delivered prematurely with respect to the negative mammary stimulation test. One patient was delivered at 34 weeks. The other two were in the thirty-fifth and thirty-sixth weeks of pregnancy, respectively, and all three of these premature infants weighed >2000 gm at birth.

Of the 16 patients with positive mammary stimulation tests, 9 gave birth to infants weighing <2000 gm, with weights ranging between 1250 and 1920 gm. Ten of the infants of these 16 women were born at ≤34 weeks' gestation.

In positive mammary stimulation tests the interval from the time of the mammary stimulation test to delivery was about 4 weeks. In negative mammary stimulation tests, this interval was approximately 9 weeks. The regimen of tocolysis was basically a standard protocol that is used on the labor and delivery unit by residents. The diagnosis of preterm labor basically requires either cervical change or documentation of uterine contractions (eight or more per hour) on the monitor. Intravenous tocolysis is accomplished by administering either magnesium sulfate or a β-sympathomimetic, such as terbutaline, followed by intravenous hydration.

We had no evidence of uterine hyperstimulation in this small series. That is probably a result of light stimulation and cessation of the test when contractions occurred. The alternative would have been to go to the end of the contraction stress test, which requires three contractions in 10 minutes and additional stimulation.[17]

The earliest that we started patients on the mammary stimulation test protocol was 24 weeks, because that is probably the earliest time at which the test can be performed, as a result of the limitations of the equipment (specifically, the sensitivity of that equipment to detect uterine contractions).

The data for twin gestations showed similar outcome measures. In fact, the two patients with twins who were delivered at term had negative mammary stimulation tests, although all patients who were delivered before term had positive mammary stimulation tests. Patients with premature rupture of the membranes had test results similar to those of patients without premature rupture of the membranes.

The incidence of preterm delivery in our patient population is related to our location. We have a very interesting patient population in the city of Detroit, as a result of the problems associated with access to prenatal care. We have approximately a 20% to 25% incidence of preterm labor or preterm delivery, or both, which unfortunately is very, very high.

We chose 5 weeks as the minimum because prenatal care usually requires that the patients be examined at 3- to 4-week intervals until late in gestation. Therefore a 5-week interval of time provides some degree of safety.

In originating the mammary stimulation test, we felt strongly that nipple stimulation was preferable to the cumbersome technique of intravenous oxytocin administration, which patients do not readily accept. However, we do agree that knowing the oxytocin levels and being able to standardize oxytocin administration are preferable.

All patients were seen after pelvic examination. In our preterm labor clinic all patients are examined by residents with staff supervision. No patient had advanced cervical dilatation, and there was minimal concern about gravidity.

A randomized trial evaluating leuprolide acetate before hysterectomy as treatment for leiomyomas

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Fifty premenopausal patients requiring hysterectomy as treatment for symptomatic uterine leiomyomas, which were the size of 14 to 18 weeks' gestation, were randomized into two groups to determine whether preoperative gonadotropin-releasing hormone agonist would increase the feasibility of vaginal rather than abdominal hysterectomy. The control group (group A; $n = 25$) did not receive preoperative gonadotropin-releasing hormone agonist, but patients in Group B ($n = 25$) received 2 months of gonadotropin-releasing hormone agonist before undergoing hysterectomy. Patients in the two groups were similar with respect to age, gravidity, parity, pretreatment uterine size, and hemoglobin and hematocrit levels. Patients in group B had an increase in hemoglobin levels (10.75 to 12.12 gm/dl, $p < 0.05$) and a decrease in uterine volume (1086.7 to 723.4 ml, $p < 0.05$) after 8 weeks of agonist therapy and were more likely to undergo vaginal hysterectomy (76.0% vs 16%). Patients in group B also had shorter hospitalizations (5.2 vs 3.8 days, $p < 0.05$). We conclude that the administration of gonadotropin-releasing hormone agonist for 2 months followed by vaginal hysterectomy is preferable to abdominal hysterectomy in selected patients with uterine leiomyomas. (AM J OBSTET GYNECOL 1991;164:1420-5.)

Key words: Leuprolide acetate, hysterectomy

When compared with abdominal hysterectomy, vaginal hysterectomy is associated with significantly fewer complications, less febrile morbidity, and the need for fewer transfusions. In addition, patients require shorter hospital stays and have a shorter convalescent period.[1] Uterine morcellation or intramyometrial coring techniques allow vaginal removal of uteri >700 gm.[2, 3] Despite the advantages of vaginal hysterectomy, some leading authorities in gynecologic surgery do not recommend vaginal hysterectomy when the uterus is larger than 10 to 14 weeks' gestational size.[4]

Many studies now show that administration of gonadotropin-releasing hormone (GnRH) agonist reduces both leiomyoma size and uterine volume and that the majority of this reduction occurs within the first 8 weeks of treatment.[5-9] The purpose of this study was to determine whether short-course preoperative GnRH agonist therapy before planned hysterectomy as treatment for uterine leiomyomas could result in increased performance of vaginal hysterectomy. We also wished to determine if this treatment would be associated with a decrease in morbidity, hospital stay, or convalescent period.

Material and methods

Premenopausal women with symptomatic uterine leiomyomas who did not desire future fertility voluntarily consented to participate in this study, which was approved by the University of Tennessee Institutional Review Board. Inclusion criteria included: (1) premenopausal status (follicle-stimulating hormone level <30 mIU/ml); (2) the absence of pregnancy or lactation; (3) the presence of fibroids 14 to 18 weeks' gestational size on pelvic examination; (4) the absence of uterine calcification on ultrasonographic examination; (5) significant symptoms including menorrhagia, pelvic pressure, or pelvic pain; (6) no suspicion of ovarian or uterine malignancy on physical or ultrasonographic examination; (7) benign endometrial histologic features (if endometrial sampling was clinically indicated).

Patients were randomized to either immediate hysterectomy (group A) or to preoperative GnRH agonist treatment (group B). Patients in group B received GnRH agonist in one of two forms according to its availability: either depot leuprolide acetate 3.75 mg (TAP Pharmaceuticals, North Chicago) intramuscularly every 4 weeks for two injections (8 treatment weeks) or

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6/6/28578

Table I. Comparison of demographic and pretreatment data between groups A and B

Characteristics	*Group A (n = 25)*	*Group B (n = 25)*	*p Value*
Age (yr)	39.5 ± 6.2 (30-51)	39.8 ± 5.6 (31-51)	NS
Gravidity	2.81 ± 2.2 (0-8)	2.69 ± 2.1 (0-8)	NS
Parity	2.41 ± 2.0 (0-6)	2.42 ± 1.9 (0-7)	NS
Pretreatment* hemoglobin (gm/dl)	11.17 ± 2.0 (8.1-15.2)	10.75 ± 2.2 (6.7-14.3)	NS
Pretreatment* hematocrit (%)	34.5 ± 4.4 (29.4-44.1)	33.2 ± 5.8 (23.4-44.0)	NS

NS, Not significant. Values are mean ± SD. Ranges are shown in parentheses.

*Pretreatment hemoglobin and hematocrit levels in group A are also the preoperative laboratory values.

0.5 mg/day subcutaneously daily for 8 weeks. Patients in group A underwent hysterectomy within 4 weeks of their randomization, and patients in group B underwent hysterectomy either 5 weeks after the last depot injection or 1 week after the last subcutaneous injection. GnRH treatment was initiated on menstrual day 2 to 4 in all patients. Blood samples for determination of serum estradiol FSH, luteinizing hormone hemoglobin, and hematocrit were obtained before treatment and at weeks 4 and 8.

Ultrasonographic measurements of fibroid and uterine dimensions were performed with an Acuson 128 machine with a 3.5 MHz transducer (Acuson, Inc., Mountview, Calif.). The uterine fundus was measured in three dimensions: anteroposterior dimension, width, and length (from internal os to top of fundus). Uterine volume was calculated by a previously described formula.[5] All patients in group B had pretreatment and posttreatment ultrasonography, and patients in group A had only pretreatment (preoperative) ultrasonography. Patients in group B underwent a bimanual examination before treatment and at weeks 4 and 8.

All patients in this study were considered eligible for vaginal hysterectomy if the uterus was mobile and was ≤14 weeks' gestational size on bimanual examination. Hysterectomies were performed by a senior gynecology resident and two authors (T.G.S., L.C.H.). All patients received a single 2 gm prophylactic dose of cefazolin before induction of anesthesia. Total blood loss was determined by weight in the following way: Total blood loss = (Blood measured from suction [milliliters] minus Irrigation used [milliliters]) plus (Wet sponge weight minus Dry sponge weight [grams]) divided by 1.06 gm/ml (Blood density). Total surgical time and operative complications were recorded. All uteri were weighed on the same scales at the time of surgery. Uterine volume was determined by water displacement at the time of surgery. Data were collected postoperatively on the number of blood transfusions, febrile morbidity, postoperative complications, length of hospital stay, and length of convalescence (time from surgery until the patient felt she could return to her normal activities).

Data were analyzed with the Student *t* test; $p < 0.05$ was considered significant.

Results

Fifty patients were eligible for study inclusion. Twenty-five patients were randomized to group A (immediate hysterectomy), and 25 patients were randomized to 2 months of leuprolide acetate before hysterectomy (group B). The two groups were similar with respect to age, gravidity, parity, and pretreatment hemoglobin and hematocrit levels (Table I).

The groups did not differ with respect to either pretreatment uterine size as measured by pelvic examination or ultrasonographically measured uterine volume (Table II). After GnRH therapy patients in group B had a reduction in uterine size from 15.7 to 11.2 weeks' mean gestational size as determined by pelvic examination ($p < 0.05$). Uterine volume on ultrasonographic measurement also decreased from a mean of 1086.7 to 723.4 ml ($p < 0.05$) after 2 months of leuprolide therapy and was not dependent on which of the two forms of the drug was used. Both preoperative uterine volume and uterine weights were lower in group B than in group A after agonist therapy, but these differences in volume and weight did not reach statistical significance (Table II).

Pretreatment and preoperative hemoglobin and hematocrit levels in group A were similar to the pretreatment levels in group B (Table I). However, patients in group B had an increase in both hemoglobin and hematocrit levels during the 2-month treatment period. Hemoglobin levels increased from a mean of 10.75 ± 2.2 gm/dl to a mean of 12.12 ± 1.1 gm/dl ($p = 0.03$), and the mean hematocrit increased from 33.2% ± 5.8% to 37.14% ± 2.8% ($p = 0.03$).

As a result of uterine size more than 14 weeks' gestation, 21 of 25 patients (84%) in group A underwent abdominal hysterectomy, although only 4 (16%) were

Table II. Comparison of uterine volume data between groups A and B

Characteristics	*Group A* (n = 25)*	*Group B (n = 25)*	*p Value*
Pretreatment uterine size (gestational wk)	15.4 ± 1.2 (14-18)	15.7 ± 1.4 (14-18)	NS
Preoperative uterine size (gestational wk)	15.4 ± 1.2 (14-18)	11.4 ± 1.6 (9-14)	0.0001†
Pretreatment ultrasonography uterine volume (ml)	888.4 ± 520.1 (356-2145)	1086.7 ± 582.4 (414-2138)	NS
Preoperative ultrasonography uterine volume (ml)	888.4 ± 520.1 (360-2145)	723.4 ± 296.9 (280-1305)	NS
Uterine weight (gm)	406.8 ± 192.5 (140-645)	341.8 ± 136.8 (175-565)	NS
Uterine volume at surgery (ml)	452.4 ± 12.2 (190-628)	365.2 ± 122.5 (140-550)	NS

NS, Not significant. Values are mean ± SD. Ranges are shown in parentheses.
*Pretreatment and preoperative values are the same for group A.
†Statistically significant.

Table III. Comparison of operative and postoperative data between groups A and B

Characteristics	*Group A* (n = 25)*	*Group B (n = 25)*	*p Value*
Operative time (minutes)	104.2 ± 29.5 (57-163)	93.1 ± 41.9 (40-145)	NS
Operative blood loss (ml)	613.9 ± 108.0 (131-1583)	527 ± 73.9 (110-1020)	0.042†
Postoperative hemoglobin at 24 hr (gm/dl)	10.0 ± 1.9 (7.6-14.1)	11.5 ± 1.3 (8.0-13.2)	NS
Postoperative hematocrit at 24 hr (%)	30.8 ± 4.4 (23.1-39.7)	32.0 ± 3.8 (21.2-36.0)	NS
Postoperative hospital stay (days)	5.2 ± 1.8 (4-11)	3.8 ± 1.9 (2-10)	0.022†
Convalescence period (days)	28.5 ± 6.2 (21-49)	17.5 ± 4.5 (10-39)	0.013†

NS, Not significant. Values are mean ± SD. Ranges are shown in parentheses.
*Pretreatment and preoperative values are the same for group A.
†Statistically significant.

considered candidates for vaginal hysterectomy. Two of four patients who underwent vaginal hysterectomy required uterine morcellation. In group B none of the patients were thought to be candidates for vaginal hysterectomy before leuprolide acetate therapy because all uteri in group B were >14 weeks' gestational size. After agonist therapy, 19 of 25 patients (76.0%) had uteri ≤14 weeks' gestational size, they were considered candidates for and underwent vaginal hysterectomy. Although all patients in this group had uteri ≤14 weeks' gestational size, four patients did not have the uterine mobility to undergo vaginal hysterectomy, and two patients experienced a failed attempt at vaginal hysterectomy and required abdominal hysterectomy. In one patient, although the uterus was small after agonist therapy, the uterus and bladder were densely adhered to the abdominal wall. This patient had an inadvertent cystotomy, which was repaired abdominally. In the other patient the cul-de-sac was obliterated with adhesions; this resulted in inadvertent sigmoid colon injury that was repaired vaginally.

There was no statistical differences in mean operative time, blood loss, or postoperative hemoglobin or hematocrit levels between patients in groups A and B (Table III). No patient required intraoperative or postoperative transfusion. However, patients in group B had significantly shorter postoperative hospital stays and convalescence periods than did patients in group A (Table III).

Comment

Selected patients with enlarged uteri that are 14 to 18 weeks' gestational size as a result of leiomyomas can safely undergo vaginal hysterectomy when GnRH agonist is used as a preoperative surgical adjuvant. This method of reducing uterine size not only is safe but also allows a greater number of patients to undergo vaginal hysterectomy. Although other authors have described removing even larger uteri vaginally,[3] in this study we used a uterine size of 14 weeks' gestation as the upper limit at which to attempt vaginal hysterectomy. Regardless of the uterine size used by the individual surgeon as a limiting factor in his or her practice, the data here suggest that preoperative agonist therapy may allow vaginal removal of a greater number of uteri.

Patients who received GnRH agonist were treated for 8 weeks before to surgery. This length of therapy was chosen because the majority of uterine size reduction occurs by approximately 8 weeks of therapy, although a small amount of reduction continues for several additional weeks.[7-8] With short-term therapy of only 2 months, there should be no concerns about bone loss, the patient can better tolerate the hypoestrogenic side effects, and the costs associated with agonist therapy are kept to a minimum.

For the patient with significant vaginal bleeding that results in anemia, this form of therapy has an additional benefit. Because the majority of patients treated with GnRH agonist therapy experience amenorrhea, concomitant iron therapy results in an increase in hemoglobin and hematocrit levels. Patients in group B demonstrated this anticipated increase. Although there was not a statistically significant difference in operative time, there was an average decrease of 71 ml of blood loss in those patients in group B when compared with those in group A. This decreased blood loss, although it is statistically significant, may or may not be of clinical significance in the selected patient.

Although uterine weights at the time of surgery in groups A and B were not statistically significant ($p > 0.05$), the difference in mean weight of 406.8 gm in group A patients and 341.8 gm in group B patients was apparently of clinical significance, because an increased number of patients in group B were able to undergo vaginal hysterectomy. Because agonist therapy resulted in a reduction both in average estimated urine size on pelvic examination from 15.7 to 11.4 weeks and in ultrasonographic volume from 1086.7 to 723.4 ml, it appears that agonist therapy may result in a greater reduction in uterine volume than in uterine weight. This hypothesis cannot be tested because uterine volume can be measured before and after treatment, but weight can be determined only after the uterus is removed.

The cost of 2 months of leuprolide acetate therapy at our institution ranges from $350 to $600, depending on whether patients are able to share the cost of a vial of drug and whether the patient chooses to use the daily injection or the depot form. This cost, however, is offset by a decreased length of hospital stay and a decreased length of convalescence, which reduce time lost from work. Future studies may show that estrogen replacement therapy can be used to decrease the hypoestrogenic side effects associated with GnRH agonist therapy without offsetting the benefits of uterine size reduction.

This study demonstrates that selected premenopausal patients with uterine leiomyomas of 14 to 18 weeks' gestational size can safely undergo vaginal hysterectomy when they are treated with a short course of leuprolide acetate before surgery. This method appears to have advantages over abdominal hysterectomy because it is associated with a shorter postoperative hospital stay and an overall decrease in the postoperative convalescent period. This technique also appears to allow time for the successful treatment of significant anemia without the risk of transfusion. The results of this study indicate that preoperative GnRH agonist therapy for 2 months before planned vaginal hysterectomy for the patient with uterine leiomyomas of 14 to 18 weeks' gestational size can be recommended.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. Bob L. SHULL, Scott & White Hospital, Temple, Texas. In 1989 Schlaff et al.[1] used a placebo-controlled trial to show that GnRH agonist therapy produced a significant reduction in total uterine volume and in nonmyoma volume but that it did not produce a significant reduction in myoma volume.[1] They did not, however, address the specific question of how much volume is too much to prevent vaginal hysterectomy.

Friedman and associates reported on their experience with a randomized, placebo-controlled, double-blind study involving leuprolide acetate before myomectomy.[2] They stratified patients by uterine volume measured by ultrasonography and showed a significant decrease in intraoperative blood loss only in the patients with ultrasonographic volumes >600 ml.

The design of this study does not include placebo controls but does include a group of untreated patients (group A). Group B, the treated group, includes patients receiving a GnRH agonist, leuprolide acetate, by one of two routes. The groups were not evaluated over the same time period and did not have the same number of clinical or ultrasonographic studies before surgery. The patients, the ultrasonographer, and the surgeon were not blinded, so that an element of bias cannot be eliminated. This study could have been strengthened by incorporating blinding or placebo procedures.

The primary thesis cannot be tested because all patients in group B were randomized not to determine whether they were candidates for vaginal or abdominal hysterectomy *before* therapy but, rather receive therapy regardless of uterine size. Reference to Table II shows that in group B uterine size varied from *12* to 18 weeks' gestational size. The authors have pointed out in the Material and methods section that all patients with uterine size ≤14 weeks' gestational size were considered candidates for vaginal hysterectomy; therefore we should conclude that at least one patient (if not more) in group B had uterine size ≤14 weeks' size before therapy and that preoperative therapy made no difference in qualifying those patients for vaginal hysterectomy as a result of uterine size only. Similarly, in group A (the untreated patients) the mean uterine size was 14.4 weeks, yet only 4 of 25 patients underwent vaginal hysterectomy. The authors provide us with no standard deviations or measures of variance; consequently, it is not possible to determine how many patients in each group should have qualified for vaginal hysterectomy as a result of initial uterine size. Nor is it possible to determine if Student's t test is appropriate for assessing significance.

To make meaningful comments about the blood loss in surgery, hospital stay, and period of convalescence in relation to the use of agonist therapy, it may be more appropriate to stratify the comparison between untreated and treated groups by the route of the hysterectomy. It is possible that agonist therapy could improve these end points irrespective of the route of surgery, but we are unable to assess this possibility with the data presented. No information correlating clinical estimates of uterine size, ultrasonographic volume determination of uterine size, and uterine weights in individual patients was provided. Such information might help us evaluate the role of these variables in clinical decision-making. I hope the authors will include their observations in a final manuscript.

A reasonable question is, what do ultrasonographically determined uterine volumes mean? In both groups A and B the uterine weights were approximately 45% of the uterine volume. Does that mean that the uterus has a density one half that of water? Using Archimedes' principle, we recently measured the volume of water displaced by extirpated uteri from 10 different patients and found that the true uterine volume closely approximates the weight of the uterus in grams. In an animal study a group of Japanese investigators determined ultrasonographic volume of the canine prostate and subsequently compared the ultrasonographic determinations with specific gravity, actual volume, and measured weights of the canine prostates determined after prostatectomy. They showed the specific gravity of the canine prostate to be approximately 1.06 and that an accurate weight could be determined by multiplying volume by specific gravity.[3]

Ultrasonographic evaluation of the pelvis may help to assess adnexal structures; however, I can find no evidence in this study or elsewhere that uterine volume measured by ultrasonography can be translated into estimates of uterine weight or gestational size. It is likely that the irregular contours of some uteri do not correspond to any single geometric formula for volume determination; therefore use of a single formula should produce an overestimation of true uterine volume, particularly in women with leiomyomas.

The authors concluded that preoperative therapy with GnRH agonist "allows selected patients *safely* to undergo vaginal hysterectomy." In group B 9% of patients who had vaginal hysterectomy attempted required abdominal completion of the surgery. In several large series approximately 1% of patients who had vaginal hysterectomy attempted required abdominal completion.[4] The current report suggests that the authors' incidence of failed vaginal hysterectomy is a significant problem that could outweigh any benefits that may be ascribed to GnRH agonist therapy.

In summary, the addition of placebo controls and the use of blinded observations would have provided meaningful data regarding the possible effects of preoperative GnRH agonist therapy. In addition, the correlation of clinical examination, ultrasonographic volume determination, and uterine weights in individual patients might have provided valuable information for the clinician who is trying to determine which patient with leiomyomas is a candidate for vaginal hysterectomy.

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DR. PEDRO A. POMA, Melrose Park, Illinois. I want to state my bias. I prefer vaginal hysterectomy if possible. If the authors are going to continue with their studies, I would like them to consider a group in whom progestin could be used. On many occasions, using progestin, which is probably one-tenth of the cost of this other fancy new product, would produce the same results.

DR. DAVID V. FOLEY, Wauwautosa, Wisconsin. I stand, not to comment on this article, although I thought it was a very meaningful one, but to challenge academicians to get back to teaching vaginal surgery.

I have been privileged over the last 14 years to be a board examiner and have been agonized by the lack of vaginal surgery that has been taught to and done by the candidates. At the last examination I examined 20 candidates. In their entire case list there were two vaginal hysterectomy cases. I asked each one of these candidates what he or she would do with a traumatic cystotomy, and not one of them could handle the situation independently.

I think that this is a travesty, and I think that we should get back to doing pelvic surgery and teaching pelvic surgery.

DR. ROBERT K. ZURAWIN, Houston, Texas. I guess that (Dr. Foley's comment) stole a little bit of the thunder from what I was going to say. The authors mentioned that in the treatment study 16 of the 19 patients who underwent hysterectomy required morcellation. That also indicates that a thorough knowledge of vaginal operative technique is necessary. When I was taught vaginal surgery, the decision to perform a hysterectomy abdominally as opposed to vaginally had to do with your clinical judgment. In other words, the success of a vaginal hysterectomy had to do with the proper selection of patients.

I did not hear any mention of whether the gravidity of the patient affected selection, nor was there any mention of pelvic anatomy, such as the narrowness of the arch, the width of the ischial spines, or those things that might also influence a surgeon to attempt a hysterectomy vaginally. But, as the previous questioners mentioned, I think that if you're going to embark on a hysterectomy with a uterus of this size, especially those that either before or after treatment are going to require morcellation, you're going to need a certain amount of surgical skill that medical treatment is not going to affect one way or the other.

DR. STOVALL (Closing). As most of you are probably aware, the United States Food and Drug Administration is currently requiring all agonist manufacturers to perform blinded placebo-controlled trials to determine the efficacy of preoperative GnRH agonist prior to myomectomy or hysterectomy. We are currently involved in three such trials, and we believe that these additional data will confirm the data presented today. It is very difficult, however, when doing clinical trials with agonists to do placebo-controlled, blinded trials because of the effects of the agonists, such as uterine size reduction, the hypoestrogenic effects with hot flashes, and vaginal dryness. Thus it is nearly impossible to perform a truly "blinded" trial.

All patients in the groups initially had uteri that were ≥14 weeks' size. The size was confirmed before treatment or surgery by two examiners. To calculate uterine volume ultrasonographically, we used the formulas that have been shown in the past to correlate gestational size with uterine volume; this was done by Sauer et al.[1] and others from several institutions.

Progestins, danazol, and other agents have been used in an attempt to reduce uterine size, but these have not been nearly as successful as GnRH agonists, or they have not been successful at all.

This study was also done in an attempt to increase the number of vaginal hysterectomy candidates at our institution so that our residents would have increased training in vaginal surgery. As the number of general gynecology patients decreases and as the indications for hysterectomy decline, we have been faced with a decrease in the number of surgical candidates for resident training, as have other institutions. An outgrowth of this has been to increase the number of vaginal hysterectomies that our residents perform.

To attempt vaginal hysterectomy for large uteri, you must be comfortable with pelvic anatomy, uterine morcellation, and other techniques used to remove large uteri. I think that more than that, you must be persistent in what you are doing and must have good operative assistance.

We believe that there are probably three roles for GnRH agonist therapy for myomas. The first is for patients who have a low preoperative hematocrit, to increase the hemoglobin and hematocrit before an intended operative procedure and thereby to decrease the chance that the patient will require either intraoperative or postoperative blood transfusions. Second, we believe that selected patients can undergo conversion from abdominal hysterectomy to vaginal hysterectomy, which decreases their overall operative and postoperative morbidity. Finally, there are patients who will undergo myomectomy who might benefit from preoperative GnRH agonist therapy. Although these patients have not been clearly identified in the literature, certainly patients who have very large uteri probably do benefit from GnRH agonist therapy before myomectomy.

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Is pulsed Doppler velocimetry useful in the management of multiple-gestation pregnancies?

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Few studies have addressed the significance of umbilical artery pulsed Doppler velocimetry in multiple gestation. Level II ultrasonography and pulsed Doppler studies were performed in 94 twin pairs and seven sets of triplets, which yielded data on 207 fetuses. A systolic/diastolic ratio was calculated for each fetus; abnormal pulsed Doppler velocimetry showed high correlation with adverse pregnancy events. Those with abnormal Doppler findings tended to be born 3 to 4 weeks earlier and to exhibit a greater number of stillbirths and structural malformations, as well as greater morbidity, when compared with fetuses without abnormal Doppler results. Fifteen of 17 infants with abnormal antenatal waveforms suffered serious morbidity. Seven were small for gestational age, and two were borderline for small for gestational age. An additional five infants with abnormal waveforms were appropriate for gestational age but were either recipient or donor in the twin transfusion syndrome. Eleven fetuses with this syndrome are described. Donor twins tended to be severely small for gestational age, with 7 of the 11 infants showing elevated systolic/diastolic ratios. Amniotic fluid volume tended to be diminished in the donor's sac but normal or increased in the recipient's sac. The observations in the study correlate with suspected physiologic changes of this syndrome. Because present findings suggest that fetuses with abnormal velocimetry suffer increased morbidity and mortality, a more rational method of management that uses Doppler data is suggested for multiple gestations. (AM J OBSTET GYNECOL 1991;164:1426-33.)

Key words: Doppler, ultrasonographic diagnosis, twins, twin transfusion syndrome, abnormalities, pregnancy

Elevated resistance in the umbilical artery as evidenced by an increased systolic/diastolic ratio has been associated with retarded fetal growth and certain fetal anomalies.[1-7] In twin gestations a large number of infants who are small for gestational age (SGA) at birth have abnormal umbilical artery Doppler findings.[8,9] Although recent information suggests that pulsed Doppler data might be of benefit in the management of twin pregnancies, the dynamic fetal cardiovascular changes associated with twin transfusion syndrome pose certain unresolved problems.[10,11] For example, Giles et al.[8] report that a number of SGA twins with twin transfusion syndrome demonstrate no intertwin difference in antenatal Doppler ratios. Pretorius et al.[11] suggest that Doppler studies in the twin transfusion syndrome did not differentiate between donor and recipient fetus in this syndrome or provide prognostic data for outcome. In a comparison of singleton pregnancies, Farmakides et al.[9] report a small increase in systolic/diastolic ratios for normal twin pregnancies and slightly lower systolic/diastolic ratios in twin fetuses with retarded growth. Therefore the relationship between abnormal Doppler values and neonatal outcomes in multiple gestations continues to demonstrate several unresolved issues.

In an effort to assess the significance of abnormal pulsed Doppler measurements in multiple gestations, an analysis is made of the umbilical artery Doppler findings, fetal ultrasonographic characteristics, and neonatal outcomes in a group of infants from such pregnancies. As a result of these findings, a potential role for pulsed Doppler velocimetry is described for multiple gestations.

Material and methods

From Jan. 1, 1987, to Jan. 4, 1989, 207 fetuses from multiple gestation pregnancies underwent analysis of pulsed Doppler waveform recordings from the umbilical artery, as well as complete level II ultrasonographic examinations. The study population consisted of high-risk patients with multiple gestations who were referred for level II ultrasonography to the Abbott-Northwestern Perinatal Center, a tertiary site. The sample included 94 twin pairs and 7 sets of triplets. Two fetuses were excluded from the sample because complete ultrasonographic data were not available. There-

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From the Perinatology Service, The Perinatal Center, Abbott-Northwestern Hospital.
Community Hospital Award, presented at the Fifty-eighth Annual Meeting of the Central Association of Obstetricians and Gynecologists, Louisville, Kentucky, October 11-13, 1990.
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Table I. Relationship between antenatal umbilical artery systolic/diastolic ratio and principal outcome variables

	Umbilical artery ratio		
	Normal systolic/diastolic ratio	*Abnormal systolic/diastolic ratio*	*p Value*
Total (N = 207)	190 (92%)	17 (8%)	
Gestational age (wk) at time of study	28.6	27.7	NS
Gestational age (wk) at delivery	33.9	30.4	0.001
Birth weight <1500 gm	19%	77%	0.001
Structural malformations	1%	12%	0.002
Apgar score ≤6 at 5 min (live-born)	8%	24%	0.018
Stillbirths	3%	12%	0.02

fore data were compiled on 207 fetuses. During the study period, 377 separate Doppler recordings and detailed ultrasonographic measurements of these pregnancies were taken. Pulsed Doppler recordings were obtained from a midsegment of fetal umbilical artery with an ATL Ultramark 8 with either a 3 or 5 MHz transducer and a sample volume size of 1.5 mm^2. Each fetus of the multiple gestation pregnancy was considered separately; the individual sac, fetus, and respective umbilical cord were identified by real-time ultrasonography. Three to five reproducible waveforms were analyzed, and a systolic/diastolic ratio was calculated; peak systolic/diastolic values were measured by calipers. Doppler values were not used for patient management decisions. For purposes of analysis only the last measurements taken before delivery were considered.

Abnormal Doppler values were assigned by comparing the value obtained in the study group with values from a normal group of 274 patients with singleton gestations matched for gestational age. Maximum and minimum normal values for systolic/diastolic ratios were etablished for each gestational age. The maximum systolic/diastolic ratios ranged from 5.0 at 16 weeks to 3.0 at 39 weeks; minimum systolic/diastolic ratio values obtained were 2.8 for 16 weeks to 2.1 at 39 weeks.

Amniotic fluid volume was considered to be increased if the greatest vertical pocket measured ≥10 cm and was considered to be decreased if the greatest vertical pocket measured ≤2 cm.

Neonatal outcome parameters included gestational age at the time of level II ultrasonography, gestational age at delivery, distribution of birth weights, presence and type of structural malformations, stillbirth, Apgar score, and delivery route. SGA infants were defined as those with a birth weight ≤10th percentile, according to Battaglia and Lubchenco.[12] Finally, the outcomes of all 16 SGA infants from the study population, as well as those of all 17 infants with abnormal Doppler values, were tabulated.

Newborns from pregnancies with twin transfusion syndrome were identified by neonatal assessment, including examination of the placenta. Newborns were then determined to be either the donor twin or the recipient twin in the syndrome; classification depended in part on the relative degree of anemia or polycythemia observed. Neonatal data used to confirm twin transfusion syndrome included disparity of newborn weight, neonatal hemoglobin and hematocrit, and microscopic examination of the placenta demonstrating a monochorionic placenta. Major neonatal morbidity, such as respiratory distress syndrome, intraventricular hemorrhage, bronchopulmonary dysplasia, fetal anomalies, and transient tachypnea of the newborn, was recorded.

The above data were tabulated and analyzed according to relationships with the umbilical artery systolic/diastolic ratio for each fetus studied. Means were calculated for the variables or groups of variables tested. Where appropriate, t tests, medians, standard deviations, and χ^2 values were calculated and compared.

Results

Table I indicates the relationship between umbilical artery systolic/diastolic ratios in multiple gestation pregnancies and principal outcome variables. Gestational age at the time of level II performance was not different between those with normal and those with abnormal systolic/diastolic ratios. However, those fetuses with abnormal systolic/diastolic ratios were born approximately 3 weeks earlier than were those with normal ratios (p = 0.001). In addition, 13 of 17 fetuses (77%) with abnormal systolic/diastolic ratios were delivered with birth weights <1500 gm, compared with 19% with similar birth weights in the normal ratio group. Structural malformations occurred in 12% of the abnormal ratio group and in only 1% of the normal ratio group. Similarly, a greater proportion of infants with abnormal antepartum ratios had a low 5-minute Apgar score, and route of delivery demonstrated no difference between systolic/diastolic ratio groups.

Sixteen of 207 fetuses (8%) were SGA at birth; Table II is a summary of Doppler findings and outcomes in each. Six cases represented twin transfusion syn-

Table II. Antenatal pulsed Doppler findings, ultrasonographic characteristics, and outcomes in 16 SGA infants from multiple gestation pregnancies

Gestational age at ultrasonography (wk)	*Umbilical arterial S/D ratio*		*Ultrasonography-estimated fetal weight percentile**	*Amniotic fluid volume*		*Gestational age at delivery (wk)*	*Birth weight (gm)*		*Comments*
	SGA twin	*AGA twin*		*SGA twin*	*AGA twin*		*SGA twin*	*AGA twin*	
31	3.0	3.1	<10th†	Normal	Increased†	31	910	1540	SGA twin: RDS AGA twin: TTS recipient, BPD
33	2.3	2.0	27th†	Normal	Normal	34	1362	2660	SGA twin: RDS AGA twin: Regular nursery
28	5.0†	2.5	<10th†	Normal	Normal	30	980	1220	SGA twin: TTS donor AGA twin: TTS recipient
23	3.8	2.6	<10th†	Normal	Increased†	24	591	763	SGA twin: TTS donor, Apgar scores 0 and 0 AGA twin: TTS recipient
28	5.3†	5.1†	27th†	Normal	Increased†	29	840	1820	SGA twin: Severe RDS, BPD AGA twin: TTS recipient, BPD, IVH
36	2.3	2.3	21st	Normal	Normal	37	1928	2668	Uneventful delivery at level I hospital
29	7.0†	2.0	<10th†	Decreased	Increased	29	902	1180	SGA twin: Apgar scores 0 and 0 AGA twin: TTS recipient, RDS, PDA, IVH
31	Reverse flow†	2.8	<10th†	Decreased†	Normal	32	760	1610	SGA twin: Coarctation of aorta, oliguria AGA twin: IVH
20	3.8	4.0	49th	Normal	Normal	38	2100	3174	Both twins: Uneventful course
22	30.0†	2.6	<10th†	Decreased†	Increased†	22	284	540	SGA twin: Stillborn AGA twin: Neonatal death
34	3.1	2.9	25th†	Normal	Normal	37	2072	2554	Both twins: Uneventful course
32	2.7	2.2	30th	Normal	Normal	37	1986	2384	Both twins: Uneventful course
29	15.1†	3.7	<10th†	Increased†	Normal	33	1052	1463	Both twins: NICU, transient tachypnea of newborn
25	4.8‡	4.2	10th†	Decreased†	Increased†	26‡	430	780	SGA twin: Stillborn AGA twin: TTS recipient, BPD, PDA
35	6.0†	2.0	27th†	Normal	Normal	37	2086	2469	Uneventful
27	3.8	3.0	52nd	Normal	Normal	37	2043	2384	Delivery at level I hospital

S/D ratio, Systolic/diastolic ratio; *RDS,* respiratory distress syndrome; *TTS,* twin transfusion syndrome; *BPD,* neonatal bronchopulmonary dysplasia; *IVH,* intraventricular hemorrhage; *PDA,* patent ductus arteriosus; *NICU,* neonatal intensive care unit.

*For smaller twin.

†Abnormal value.

‡Borderline high Doppler value.

drome by neonatal assessment. This diagnosis was supported by placental examination and by findings of diamnionic-monochorionic placentation. In every case amniotic fluid volume was normal or decreased in the donor twin, whereas five of six recipient twin fetuses showed hydramnios; only one recipient had a normal fluid volume. The umbilical artery ratio was elevated in four of the six donor twins and was borderline in one twin, but it was elevated in only one of the recipient twins. In general, the degree of retarded fetal growth

Table III. Outcome of 17 infants from multiple gestations with abnormal antenatal pulsed Doppler umbilical artery systolic/diastolic ratios

Systolic/diastolic ratio	*No. of fetuses*	*Gestational age* (wk)*	*Neonatal outcome*
5.0, 5.3, 7.0, reverse flow, 30, 15.1, 6.0	7	22, 23 37, 30 29, 29 32	SGA (<10th percentile; Table II)
5.2, 4.9	2	31, 31	Borderline SGA (birth weight between 10th and 20th percentiles for gestational age); NICU admission
10.0	1	29	Gastroschisis; moderate respiratory distress syndrome; grade III/IV intraventricular hemorrhage; persistent patent ductus arteriosus
4.4, 5.0, 5.0, 4.8, 5.1	5	26, 26, 27 29, 31	Twin transfusion syndrome (3 donors and 2 recipients): 1 borderline SGA (donor); 1 survivor of triplet pregnancy (donor); (2 other fetuses died in utero); 1 AGA (donor) (neonatal death); 1 recipient with intraventricular hemorrhage and ischemic encephalopathy (69-day NICU stay; 1 recipient with moderate respiratory distress syndrome and persistent patent ductus arteriosus
6.6	1	37	Other twin of pair with anencephaly; both with abnormal Doppler ratios; survivor had uneventful course
4.4	1	37	Borderline abnormal S/D ratio at 34 wk; uneventful course

NICU, Neonatal intensive care unit.
*At time of delivery.

tended to be profound in the donor twin. Three of the donor twins were stillborn, and the rest suffered significant neonatal morbidity. All except two of the infants had one or more abnormalities of amniotic fluid volume, pulsed Doppler values, or ultrasonographically estimated fetal weight at ≤35th percentile. In both cases these twins were born uneventfully at 37 and 38 weeks, respectively, at a level I hospital.

Table III illustrates the outcomes in the 10 fetuses who showed abnormal pulsed Doppler findings antenatally but were not SGA. Two were between the 10th and 20th percentiles for birth weight and missed the SGA classification by only 150 gm. Five were appropriate-for-gestational-age (AGA) fetuses (three donor and two recipient twins) involved in the twin transfusion syndrome. One exhibited major congenital anomalies, including gastroschisis and ventricular septal defect, and one twin was opposite a sibling with anencephaly (both showing elevated systolic/diastolic ratios). Finally, one twin had a borderline elevated ratio of 4.4 at 34 weeks; he was appropriate-for-gestational-age without apparent morbidity. With the exception of that infant, all remaining AGA twins with abnormal ratios were admitted to the neonatal intensive care unit; the recipient twin in the twin transfusion syndrome suffered serious morbidity.

Amniotic fluid volume tended to be increased or to show hydramnios in 16% of the multiple gestation sample. This relationship held for those grouped according to umbilical artery ratio and birth weight status (SGA vs non-SGA) at p values of 0.01 and 0.06, respectively. Amniotic fluid volume tended to be decreased in the donor twin and increased in the recipient twin, as noted.

Comment

The numbers of patients in this study and their attendant complications permit sufficient opportunity to infer the potential role of pulsed Doppler velocimetry and real-time ultrasonography in the management of such pregnancies.

This article suggests that patients with abnormal Doppler findings have a significant number of other adverse pregnancy complications, some of which are unique to multiple gestations. Of the 17 abnormal Doppler systolic/diastolic ratios in the study group, 7 were associated with an SGA infant. Of the remaining 10, all infants except two were admitted to the neonatal intensive care unit. Five AGA infants (three donor and two recipient fetuses) were products of pregnancies complicated by twin transfusion syndrome. All members of this group either died in the neonatal period or had prolonged, complicated neonatal courses. Of the remaining five, two were borderline SGA, with birth weights ≤20th percentile, and one was affected with gastroschisis and ventricular septal defect. Thus 15 of 17 fetuses with abnormal Doppler velocimetry had adverse perinatal outcomes.

In addition, those fetuses with abnormal Doppler systolic/diastolic ratios were born 3 to 4 weeks earlier ($p = 0.001$) than those fetuses with normal ratios. Fetuses with abnormal waveforms were much more likely to have structural malformations (12% vs 1%), to be <1500 gm in weight, to have lower 5-minute Apgar scores, and to suffer intrauterine fetal death (12% vs 3%).

Several investigators suggest that Doppler studies may be useful in evaluating retarded fetal growth in twin pregnancies. Farmakides et al.[9] reported slightly lower systolic/diastolic ratios in growth-retarded twins when compared with singletons. Although our study did not specifically address this issue, review of Table II indicates that numerous SGA twins exhibited markedly abnormal waveforms, including reversal of diastolic flow.

The present investigation demonstrates experience with 11 fetuses with documented twin transfusion syndrome. Giles et al.[8] reported in their article on twin pregnancies, "We believe that twin-to-twin transfusion may be strongly suspected if a discrepancy in ultrasound size is seen in the presence of no A/B (S/D) ratio difference." Our article does not support this position. Although both recipient and donor twins may have normal velocimetry, the most consistent observations indicate that the donor (usually an SGA fetus) will show abnormal systolic/diastolic ratios, as did 7 of 11 donor fetuses. Normal systolic/diastolic ratios were most frequently seen in the recipient twin, although abnormal ratios were noted in 2 of 10 recipient fetuses. These observations agree with those of Pretorius et al.,[11] who noted varying Doppler findings in both donor and recipient fetuses in the twin transfusion syndrome. Commonly, the donor fetus showed normal or decreased amniotic fluid volume, whereas the recipient fetus indicated signs of both hydrops and hydramnios.

Unbalanced arteriovenous shunting appears to be the major physiologic event in the twin transfusion syndrome. The donor twin becomes progressively anemic and hypovolemic and experiences a consequent decrease in umbilical blood flow. The resultant increase in umbilical vascular resistance accounts for the present observations, which were noted predominantly in donor twins of increased systolic/diastolic ratios; this reflects increased resistance to flow. Oliguria was a clinical problem for the neonatologists in many of the profoundly SGA donor twins, and decreased amniotic fluid volume was a frequent observation; both of these support the hypothesis for the previously described sequence of events. Our findings, which are in agreement with those of Pretorius et al.,[11] support the concept that twin transfusion syndrome is a complex pathophysiologic event. However, Doppler interrogation of the umbilical artery also may permit study of the spectrum of change and severity in this altered hemodynamic state.

The role of real-time ultrasonography in the management of multiple gestation pregnancy recently has received increased attention. D'Alton and Mercer[13] indicate that significant reductions are possible in preterm delivery rates and in the incidence of perinatal deaths when real-time ultrasonography is used systematically as an adjuvant to routine prenatal care.

Our data suggest that fetuses from multiple gestations with abnormal umbilical artery systolic/diastolic ratios suffer a disproportionate increase in serious short- and long-term neonatal morbidity. For singleton pregnancies, both Berkowitz et al.[6] and Rochelson et al.[7] reported that abnormal umbilical artery velocimetry is valuable in identifying fetuses at risk for adverse perinatal outcomes. Our data also suggest a potential role for pulsed Doppler fetal velocimetry when combined with other real-time ultrasonographic measurements in the clinical management of multiple gestations.

Because morbidity and mortality occurred principally in the 8% of multiple gestation pregnancies with abnormal Doppler values, Doppler screening and real-time measurements (performed at 24 to 26 weeks' gestation, for example) could be used to identify those pregnancies most at risk for adverse outcomes. Those pregnancies showing concordant fetal growth and normal Doppler values would require less frequent ultrasonographic examinations and tests of fetal well-being. Fetuses demonstrating abnormal velocimetry could be placed in a program of more intense fetal surveillance. Thus perinatal resources could best be directed to the small number of fetuses at risk for significant morbidity and could reduce the frequency of testing for the lower-risk group. Future clinical studies are needed to assess the validity of these observations and to originate a specific program of testing and follow-up that is based on the results of Doppler data.

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Discussion

DR. PAUL G. TOMICH, Maywood, Illinois. Dr. Gaziano has investigated the usefulness of Doppler velocimetry in the management of multiple pregnancies. Two hundred seven fetuses (94 twin pairs and 7 sets of triplets) were studied. There were 190 normal studies and 17 abnormal studies. The results of these studies were not used for patient management. Neonatal outcome parameters were tabulated and analyzed and the data provided give us the "natural history" of those infants with abnormal Doppler results. Those 17 fetuses were statistically at increased risk for adverse perinatal outcome, including earlier birth, birth weight <1500 gm, major anomaly, low Apgar score, and stillbirth. Fifteen of the 17 fetuses had significant perinatal morbidity.

Much of the prenatal surveillance activity in twin pregnancies is driven by the concept of discordance, which was popularized by Babson[1] in 1964. With growth disparity, the differential diagnosis includes intrauterine growth retardation and the twin-twin transfusion syndrome. In the past obstetricians did not have a useful tool with which to screen twin pregnancies for adverse outcome; they relied on serial ultrasonographic examinations to assess potential fetal problems. Doppler ultrasonography, which was introduced as a clinical tool in the mid-1980s, would seem to be the ideal surveillance tool for multiple pregnancies.

Five previous studies have investigated the role of Doppler studies in the management of twin pregnancies.[2-6] Four of these studies have demonstrated good sensitivity (76%), specificity (83%), and positive predictive value (77%) for detecting delayed or adverse outcome. The study of Hastie et al.,[6] with a strict definition of SGA, had a sensitivity of 29%, a specificity of 88%, and a positive predictive value of 34%.

The value of velocimetry in the diagnosis of the twin-twin transfusion syndrome has not yet been established. Farmakides et al.[2] suspected twin-twin transfusion syndrome when growth discordancy or significant systolic/diastolic differences occurred. Pretorius et al.[7] reported variable Doppler findings in twin pregnancies with the twin-twin transfusion syndrome. Dr. Gaziano has identified six pregnancies with twin-twin transfusion syndrome. Four of the six donors had elevated systolic/diastolic ratios. Five of the recipient twins had normal systolic/diastolic ratios.

Gaziano and colleagues suggested that Doppler screening at 24 to 26 weeks' gestation would detect those twin pregnancies at high risk for adverse perinatal outcome, so that a more intensive fetal surveillance program could be initiated to improve outcome.

In my opinion, pulsed Doppler velocimetry is still in the investigational stage for the management of twin pregnancies. Further clinical studies are necessary before its use is considered as "standard of care" for twin pregnancies.

I have the following questions for Dr. Gaziano: (1) As a result of this study, what is your current management plan for patients with multiple pregnancies? (2) If a patient has an abnormal Doppler study, how is her management altered? (3) How are you managing patients who have ultrasonographic or Doppler studies that are suggestive of or diagnostic of the twin-twin transfusion syndrome? Are you doing percutaneous umbilical blood sampling?

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DR. NEIL K. KOCHENOUR, Salt Lake City, Utah. Doppler velocimetry is a recently developed addition to the armamentarium for antepartum fetal assessment. A number of investigators have demonstrated that an abnormal waveform is indicative of a poor perinatal outcome. General agreement exists that the chances of having a jeopardized fetus are increased in the presence of an abnormal waveform. The important question is, is this clinically useful and, if so, when and in what patient populations?

In this article the authors adopted the Doppler waveform as a method of evaluating twin gestations and asked the question, is pulsed Doppler velocimetry useful in the management of multiple gestation pregnancies? Unfortunately, I do not believe that these authors answered their own question. Here, as in the vast majority of literature concerning Doppler velocimetry, a poor outcome is correlated with the presence of an abnormal waveform. The authors demonstrated that fetuses with abnormal Doppler findings tend to be born early, to have a greater number of stillbirths and structural malformations, and to have greater morbidity than do those fetuses with normal waveforms. They did not show that this form of evaluation is helpful in improving the management of patients with twin gestations. For the purposes of their article they used only the measurement taken closest to delivery. I am interested in knowing whether changes occurred in the Doppler flow pattern in patients with serial follow-up. If so, did normal values change to abnormal ones, or did abnormal values change to normal?

Because the authors used only one value in their analysis, are they advocating the use of Doppler as a one-time screening tool or are they advocating its use for serial evaluation? If they advocate a one-time use, when should this be done? They suggest 24 to 26 weeks' gestation, but I do not see any evidence to support this interval. In fact, the mean gestational age at the time of the Doppler evaluation in this series was 28 weeks.

I have a particular interest in the twin-twin transfusion syndrome. The existing literature on this topic regarding umbilical artery Doppler velocimetry is inconclusive. These authors do not support the findings of Giles et al.,[1] who found that a discrepancy in ultrasonographically estimated fetal weight in the presence of no fetal systolic/diastolic ratio difference was indicative of fetal transfusion. Pretorius et al.[2] found an intertwin difference of 0.4 in all eight cases of twin-twin transfusion. In at least three patients with twin-twin transfusion in this series, the systolic/diastolic ratio difference was <0.4. This study found abnormal systolic/diastolic ratios in 7 of 11 donor fetuses and normal systolic/diastolic ratios in the majority of recipient twins. How do the authors recommend using Doppler to diagnose the twin-twin transfusion syndrome, and how did they differentiate this syndrome from other causes of discordant growth? The authors state that the diagnosis was made by neonatal assessment, including examination of the placenta. What were the criteria for this diagnosis?

The authors state that 77% of the fetuses with an abnormal systolic/diastolic ratio were born with a birth weight of <1500 gm, compared with 19% of the normal ratio group. Because those with abnormal Doppler results were also born 3 weeks early, I believe that these data should be normalized for gestational age.

The authors report that a greater proportion of infants with abnormal antepartum ratios exhibited low 5-minute Apgar scores. What is the significance of an Apgar score when the average age at birth was 30 weeks for the abnormal infants and 33 weeks for the normal infants? Were there differences in cord blood gases?

The authors state that all of the 16 SGA fetuses but two had abnormalities of amniotic fluid volume or pulsed Doppler ratio or an ultrasonographically estimated fetal weight of <35th percentile. What is the significance of the 35th percentile, and why was it chosen? Are the authors suggesting that twins with an estimated fetal weight of <35th percentile are growth-retarded? Is Doppler velocimetry a better predictor of the SGA fetus than ultrasonographically estimated fetal weight? Were the structural abnormalities in the fetuses with abnormal waveforms ascertained with ultrasonography? If so, what was the value of the abnormal Doppler results in these fetuses? The authors state that in their study morbidity and mortality occurred principally in the 8% of multiple gestations with abnormal Doppler values. They conclude that this may identify those pregnancies that are at higher risk for adverse outcomes. I do not dispute this. However, how is the Doppler velocimetry used when a risk is identified? Is Doppler velocimetry better than existing methods of antepartum assessment for multiple gestations?

In summary, I believe that these authors have confirmed the findings of others. An abnormal waveform is associated with a poor perinatal outcome. However, they have not, to my satisfaction, demonstrated that the use of Doppler velocimetry improves the pregnancy outcome, which is, after all, the purpose of antepartum fetal assessment.

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Dr. David B. Schwartz, Detroit, Michigan. I enjoyed the article. We have a particular interest in multiple pregnancies and have a specific multiple pregnancy program at our hospital. Most of my questions have been asked by the discussants, but I have two additional questions for Dr. Gaziano. I was unclear whether other fetal resistance and flow parameters were examined. If they were, was "centralization" of fetal circulation, such as the increased cerebral velocity or the decreased resistance that may be expected to occur with uteroplacental insufficiency, of help?

Second, I was unclear about the discordancy criteria that were used in the regular ultrasonographic examinations. Did the discordancy results correlate with the Doppler data, and would they have been of equal predictive value in these cases?

Dr. Gaziano (Closing). I appreciate the comments, and I'll try to address them in order.

First, I think it's very important that this presentation be viewed as one in which morbidity was sufficiently observed in multiple gestations with abnormal Doppler results and that this morbidity might serve in the future as a predictor or be useful in management.

In no way should you walk away from this presentation believing that Doppler velocimetry is the answer to the management of twin gestation for morbidity or mortality. I believe that Doppler velocimetry provides another piece of useful information, but we'll go through some of the specifics.

Do we routinely do Doppler examinations? Doppler examinations are part of our level II ultrasonographic examinations. We presently look at the Doppler values, but they are not the final decision-making factors for us. If we see an extremely abnormal Doppler value, such as reversal of diastolic flow, we may act on that information. We do not do cordocentesis for twin gestation.

The 24- to 26-week gestational age was used to create a hypothesis for future studies and was based on the possibility that intervention or changes in management might be effective at that gestational age.

With respect to the twin-to-twin transfusion observations in the literature, first of all, the study by Giles et al. was done with continuous-wave Doppler, and our study was done with pulsed Doppler. Continuous-wave Doppler is a blind approach. This article, and also the work by Pretorius et al., disagreed with the initial observation in the study by Giles et al., which is that there were no differences in Doppler in the twin-to-twin transfusion syndrome.

We did not obtain cord gas values. The significance for Apgar scores is simply another observation, in that overall morbidity was increased in the group with abnormal Doppler values.

Structural malformations were diagnosed antenatally by level II ultrasonographic examinations.

There were several questions related to the relationship between Doppler as a predictor of SGA babies and Doppler as a factor in predicting morbidity and mortality.

From similar data we have demonstrated that Doppler is no better than estimated fetal weight in the prediction of the SGA baby, but the point of this article is that if the Doppler value is abnormal, morbidity and mortality appear to be increased. The SGA fetus with normal Doppler values tends to have better outcomes compared with the SGA fetus with abnormal values (see Table II). For skeptics it is such observations that may confirm Doppler's unique management role.

Again, finally, let me conclude by saying that the purpose of the article was not to present strict guidelines regarding management but to suggest that the findings are significant enough to warrant prospective studies. Our belief, based on the present data, is that pulsed Doppler technology will be a useful management tool.

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Comparative efficacy of umbilical arterial Doppler indices for predicting adverse perinatal outcome

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Kansas City, Missouri

A variety of descriptor indices for the umbilical arterial Doppler waveform are currently in use for predicting perinatal outcome. However, there is a paucity of data regarding the relative diagnostic merits of these indices. We addressed this issue in a prospective, blind study in which the diagnostic efficacy of the following Doppler indices was compared: resistance index, systolic/diastolic ratio, pulsatility index, and diastolic average ratio. The analytic technique consisted of the receiver operating characteristic method, which evaluates a test's ability to discriminate a diseased from a healthy population. The patient population consisted of 350 pregnant patients with gestational ages of 34 to 36 weeks. A continuous-wave Doppler device with a 4 MHz transducer was used to investigate the umbilical vessels. The test was performed only once in each patient. The abnormal outcome parameters included small for gestational age (<10th percentile), Apgar score <7 at 5 minutes, fetal distress (late and severe variable decelerations, absent variability, fetal scalp pH <7.20), umbilical cord arterial pH <7.10, presence of thick meconium, and admission to neonatal intensive care unit (>48 hours). The results showed that the resistance index had the best discriminatory ability when compared with other Doppler indices: the systolic/diastolic ratio ($p < 0.05$), the pulsatility index ($p < 0.001$), and the diastolic/average ratio ($p < 0.05$). It is concluded that of the various Doppler indices assessed in this investigation, the resistance index offers the best diagnostic efficacy in predicting perinatal compromise. (AM J OBSTET GYNECOL 1991;164:1434-40.)

Key words: Doppler indices, comparison, perinatal outcome, receiver operating characteristic

As the Doppler frequency shifts in a circulation reflect the velocity of blood flow, the analysis of the Doppler waveform can yield information on the hemodynamic condition of a circulatory system.[1] A number of descriptor indices for the Doppler waveform have been developed for general vascular and fetal applications.[2-5] Although it has been demonstrated that changes in the umbilical arterial Doppler indices can be associated with various pregnancy complications and can be diagnostic of fetal compromise,[6-9] there have been few investigations examining the comparative efficacy of these indices. Moreover, most of the existing studies are based on threshold values derived from the test's distribution attributes. The limitations of this approach are well recognized.[10, 11] The technique of receiver operating characteristics analysis is free from these limitations, as it enables comprehensive evaluation of a test's inherent discriminatory power to separate the compromised from the healthy population.[11, 12] This analytic approach was used in a prospective blinded study, which was designed to assess the diagnostic efficacy of the umbilical arterial Doppler indices for predicting adverse perinatal outcome in an indigent population. Out of this project, we have previously reported analyses of the discriminatory powers of the umbilical arterial systolic/diastolic ratio[13] and resistance index.[14] This concluding article compares the diagnostic efficacy of the various Doppler indices, with the use of receiver operating characteristic analyses for evaluating two tests performed on the same population.[15]

Material and methods

Patient population. The patient population, as briefly described here, is the same as reported earlier.[13] A total of 350 pregnant women with gestational ages from 34 to 36 weeks participated in the study. Most were medically indigent and had a high preponderance of adverse perinatal outcome. The study was conducted with the approval of the institutional review board, and an informed consent was obtained from each patient. The perinatal outcome parameters are as follows: small for

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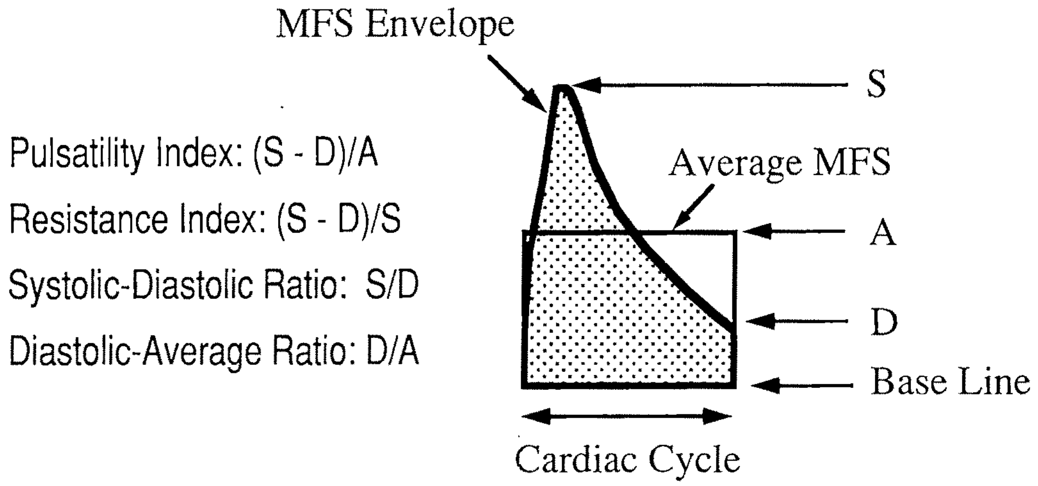
MFS Envelope
Pulsatility Index: (S - D)/A
Resistance Index: (S - D)/S
Systolic-Diastolic Ratio: S/D
Diastolic-Average Ratio: D/A
S
Average MFS
A
D
Base Line
Cardiac Cycle

Fig. 1. Graphic depiction of Doppler indices. *S*, Peak systolic value of maximum frequency shift envelope; *A*, average value of maximum frequency shift over cardiac cycle; *D*, end-diastolic value of maximum frequency shift envelope; *MFS*, maximum frequency shift.

Table I. Distribution of abnormal outcome cases

	No. of cases	%
Small for gestational age (weight <10th percentile)	43	39.4
Apgar score <7 at 5 min	9	8.3
Umbilical arterial pH <7.1	5	4.6
Fetal distress	36	33.0
Meconium	52	47.7
Neonatal intensive care unit	5	4.6
Total abnormal outcomes	107*	

*An individual case may have one or more abnormal outcomes.

gestational age (<10th percentile), Apgar score<7 at 5 minutes, umbilical arterial (cord) pH <7.10, fetal distress (late decelerations, severe variable decelerations, absent variability, fetal scalp pH <7.20), presence of thick meconium, and admission to the neonatal intensive care unit (>48 hours).

Doppler procedure. The technique of Doppler examination has been described earlier. It should be noted that the examination was performed only once on each patient. Four Doppler indices (Fig. 1) were used in this comparison: the systolic/diastolic ratio (also known as the A/B ratio),[2] the resistance index[5] the Gosling peak-to-peak pulsatility index,[3] and the normalized end-diastolic frequency shift, described as the diastolic/average ratio.[4]

Data analysis. Comprehensive receiver operating characteristic techniques were used for data analysis.[14] These included visual analysis of the receiver operating characteristic curve on the basis of the measured test values, and determination of the optimal cutoff point and determination of area under the curve by use of trapezoidal and curve-fitting methods. The latter included the binormal graph technique as described by Swets and Pickett[15] and the exponential receiver operating characteristic curve-fitting method of England.[16] In this study, performances of the various Doppler indices obtained from the same patient population were evaluated with the use of areas under the receiver operating characteristic curve derived from the above techniques that also estimated the areas un-

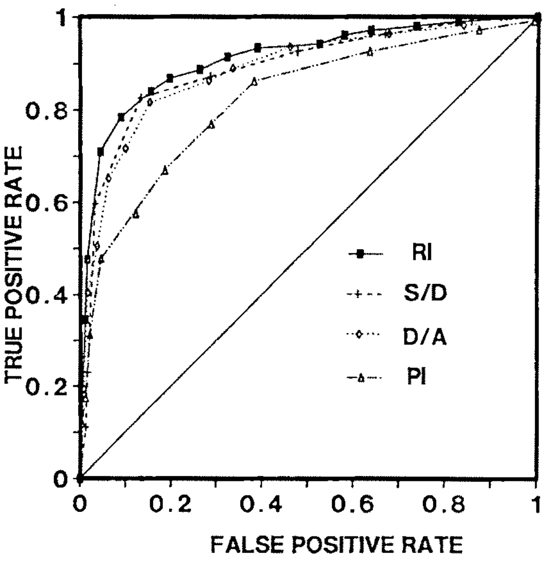

Fig. 2. Receiver operating characteristic curves of umbilical arterial Doppler indices. Data points are measured values of indices.

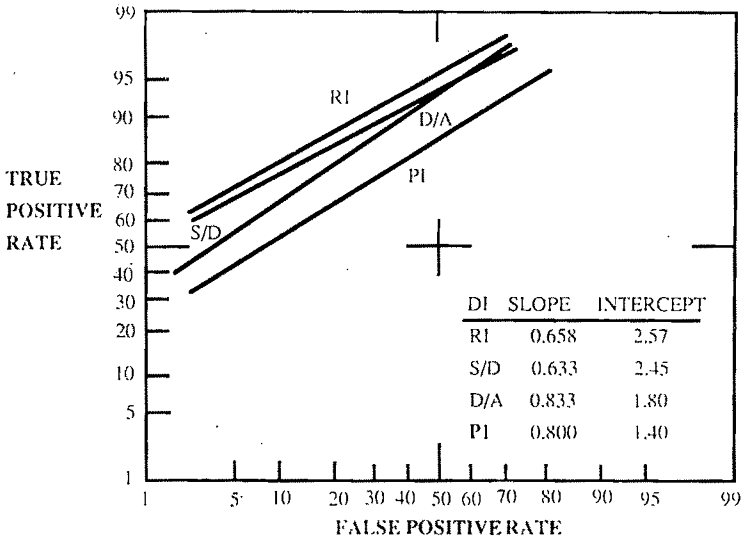

Fig. 3. Swets binormal receiver operating characteristic graphs of Doppler indices. Inset depicts slope.

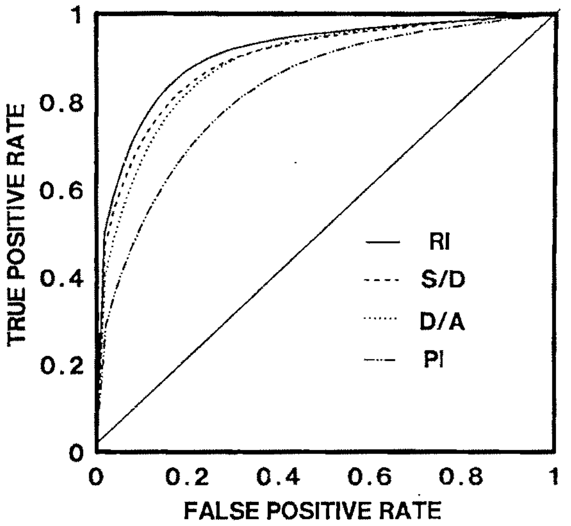

Fig. 4. Exponential receiver operating characteristic curves of umbilical arterial Doppler indices. Exponential *(E)* and receiver *(R)* values of indices were as follows: resistance index *(RI)*, E = 10.35; R = 0.629; systolic/diastolic ratio *(S/D)*, E = 8.621; R = 0.661; diastolic/average ratio *(D/A)*, E = 7.916; R = 0.555; pulsatility index *(PI)*, E = 4.797; R = 0.554.

der the curves and their standard errors. The method of Hanley and McNeil[17] was used for determining whether the differences in the areas under the curves of the four Doppler indices were random or real. The method essentially consisted of computing a ratio Z using the equation:

$$Z = (A_1 - A_2)/(SE_1^2 \times SE_2^2 - 2 \cdot r \cdot SE_1^2 \cdot SE_2^2)$$

where A_1 and A_2 are the areas obtained from the receiver operating characteristic curves from two indices, SE_1 and SE_2 are the corresponding standard errors, and r is the estimated correlation coefficient between A_1 and A_2. The latter was determined with Pearson product moment correlation, as the data in this study were of continuous scale.[18] The Z values were estimated for the various combinations of the indices, and the statistical significance (p values) was determined from the normal distribution table. The results were considered statistically significant if p was <0.05.

Results

The perinatal outcome data are presented in Table I. This is identical to our previously published report on the diagnostic efficacy of the systolic/diastolic ratio,[13] except for the cord arterial pH threshold, which was selected at 7.10. Fig. 2 depicts a visual comparison of the receiver operating characteristic curves based on the measured values of all the Doppler indices investigated in this study. As apparent, the resistance index and systolic/diastolic and diastolic/average ratios clustered closely and away from the pulsatility index. The latter was nearer to the diagonal line, indicating less inherent diagnostic efficacy than the other indices. Fig. 3 depicts the binormal receiver operating characteristic graphs of the Doppler indices as determined by the technique of Swets and Pickett.[15] It shows that the resistance index, systolic/diastolic ratio, and diastolic/average ratio cluster higher than the pulsatility index along the negative diagonal of the binormal graph, and the resistance index is located highest. The comparative exponential receiver operating characteristic curves of the Doppler indices are shown in Fig. 4. The estimated areas under the receiver operating characteristic curves and the corresponding standard errors derived by the various techniques are presented in Table II. As is evident, of all the indices the resistance index demonstrated the largest and the pulsatility index the least

Table II. Receiver operating characteristic areas and their standard errors of the Doppler indices

	Resistance index		*Systolic/diastolic ratio*		*Pulsatility index*		*Diastolic/average ratio*	
Model	*ROC area*	*SE*	*ROC area*	*SE*	*ROC area*	*SE*	*ROC area*	*SE*
Trapezoidal	0.910	0.0183	0.888	0.0204	0.822	0.0251	0.885	0.0206
Swets[12]	0.921	0.0211	0.905	0.0193	0.808	0.0234	0.887	0.0292
Exponential receiver operating characteristic	0.912	0.0175	0.896	0.0190	0.828	0.0237	0.888	0.0192

Table III. Statistical comparison of the receiver operating characteristic areas of the Doppler indices

	Resistance index			*Systolic/diastolic ratio*		
Item	*vs Systolic/diastolic ratio*	*vs Pulsatility index*	*vs Diastolic/average ratio*	*vs Pulsatility index*	*vs Diastolic/average ratio*	*Pulsatility index vs diastolic/average ratio*
R	0.870	0.701	0.711	0.675	0.731	0.411
Z	1.702	4.951	1.706	3.831	0.571	2.538
p Value	<0.047	<0.001	<0.046	<0.001	<0.284	<0.006

R, Correlation between indices; *Z*, critical ratio of normal distribution.[18]

receiver operating characteristic areas. Table III shows the statistical comparison of the receiver operating characteristic areas of the indices. The results demonstrate that the resistance index has the best discriminatory ability as compared with the systolic/diastolic ratio ($p < 0.05$), the diastolic/average ratio ($p < 0.05$), and the pulsatility index ($p < 0.001$). The systolic/diastolic ratio is superior to the pulsatility index ($p < 0.001$) but is not statistically better than the diastolic/average ratio ($p < 0.284$). Finally, it was observed that the performance of the diastolic/average ratio was statistically superior to the pulsatility index ($p < 0.006$).

Comment

Umbilical arterial Doppler indices show immense potential as a fetal surveillance tool. A number of indices have been described, but their relative merits or capabilities remain largely unsubstantiated. It may be argued that most of the indices express the pulsatility, which is the differential between the peak systolic and the end-diastolic values of the Doppler waveform; therefore there may not be any significant differences between their diagnostic efficacy. Indeed, all the indices demonstrate a high degree of correlation. However, there are computational differences in the indices that demonstrate varying distribution characteristics. The situation has been further complicated by the fact that various investigators have shown diverse preferences in choosing an index. Whereas some prefer the systolic/diastolic ratio, others continue to recommend the resistance index or the pulsatility index. Obviously, any choice must be based on the demonstrated diagnostic superiority of one index over the others.

Although such comparative efficacy can be investigated in terms of traditional test performance parameters of sensitivity, specificity, etc., these are dependent on the selection of distribution-related single threshold values. Limitations of such procedures have been recognized, especially since they do not take into account the overall performance of a test in discriminating between the diseased and the nondiseased population. To mitigate these deficiencies, the receiver operating characteristic–based analytic procedures have been recommended for evaluating a test.[18] These techniques have been used extensively in diagnostic radiology[11] and to a limited extent in evaluating the Doppler indices for fetal applications.[8, 13] Because of the differing clinical and technical circumstances, it may not be justifiable to recommend a specific test value as the universally applicable optimal operating point. As pointed out by Swets,[18] a fitted receiver operating characteristic curve, in contrast, represents all possible decision criteria as continuous data and is, therefore, independent of any single source of bias; the area under the receiver operating characteristic curve offers a general index of accuracy independent of other factors such as the population attributes or prevalence rates or the intended use of the test as a screening or a diagnostic tool.[11] Therefore the comparison of areas under receiver operating characteristic curves would allow reaching far more rational and convincing conclusions regarding diagnostic efficacy of tests than would be possible from a comparison of performances at a single operating point.

This analytic approach has been further extended to develop a method of statistical comparison of two tests performed in the same population.[18] In this study, the areas under the receiver operating characteristic curves of the various indices have been estimated by three methods. The rationale is as follows. The trapezoidal method is based on the measured receiver operating characteristic data points. However, this method underestimates the true area under the curve. Swets' graphic method of estimating the area under the receiver operating characteristic curve is considered to be the standard[11] and is most widely used. However, it approximates the receiver operating characteristic area, and, although it is considered to be a robust technique even when the distribution is not known,[17] it has an inherent assumption of Gaussian distribution of the test values.[15] Therefore application of this technique may not be appropriate for analyzing data such as the systolic/diastolic ratio and pulsatility index, which are known to have non-Gaussian distribution.[19] To ensure appropriateness of the analytic method, the receiver operating characteristic areas and standard error values were also estimated by the exponential model, which is free of any distribution assumptions about the underlying data. As evident, the results generated by Swets' graphic method are comparable to those obtained by the exponential receiver operating characteristic method confirming the applicability of the former, even when the data are known to be non-Gaussian in distribution.

Our results show discriminative differences between the various indices. Of all the indices, the resistance index demonstrated the largest and the pulsatility index the least receiver operating characteristic areas. Moreover, statistical analyses of the areas derived from the fitted curves and from the trapezoidal method show statistically significant superiority of the resistance index over the other indices. In contrast, the pulsatility index demonstrated the least diagnostic ability. The results of this study should be interpreted in the context of its practical implications. Although the diagnostic ability of the umbilical arterial Doppler indices in predicting fetal growth retardation has not been impressive,[13, 20] it has shown a significant potential in one area of fetal diagnoses. Several preliminary studies indicate that the technique, especially as applied to the umbilical arterial circulation, can be a powerful predictor of adverse perinatal outcome.[7, 8, 21] To assess further the utility of the Doppler technique as a fetal surveillance tool, it is important to standardize the technique. This includes selection of a descriptor index that would be computationally simple and yet diagnostically superior. Although the systolic/diastolic ratio has been used extensively in this country, its efficacy has not been compared with that of other indices. The resistance index enjoys the same degree of computational simplicity as the systolic/diastolic ratio, because both are based on the peak systolic and the end-diastolic values of the Doppler waveform. In contrast, the computation of pulsatility index and diastolic/average ratio is more complex and may be more prone to error, because it requires estimation of the area under the Doppler wave. To its advantage, the resistance index, unlike the systolic/diastolic ratio, demonstrates a statistically significant Gaussian distribution,[19] which facilitates its statistical manipulations. However, the most relevant point is the inherent discriminatory efficacy of the resistance index as a diagnostic test. Because of this, its use should be preferred for assessing umbilical arterial hemodynamics. Future studies will address the same issue in regard to repetitive testing in the antepartum period for predicting fetal compromise.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Daniel A. Rightmire, Springfield, Illinois. This article is of great interest to all who follow the developments of obstetric Doppler, because each of us has our favorite Doppler index. Coming after the demonstration of the non-Gaussian characteristics of the systolic/diastolic ratio and the Gaussian distribution of the resistance index, this article presents the second strong, rational argument for the standardization of the reporting of obstetric Doppler data in the resistance index format. The exception would appear to be for those studies or cases in which diastolic Doppler frequency shifts are lost below the high-pass filter or are actually reversed.

I would like to ask Dr. Maulik a few questions. First, what have you determined to be the best threshold value of the resistance index in your study? Also, what statistical method did you use to find that optimum decision threshold?

Second, now that you have shown us the receiver operating characteristic curves, why should we not continue to use systolic/diastolic or diastolic/average threshold values, which are comparable to the best resistance index value for predicting poor pregnancy outcome? Surely the systolic/diastolic value of 3.0 that has been widely publicized as the threshold of abnormal waveforms in third-trimester umbilical artery studies seems to be as good a discriminator as any resistance index value.

I would like to point out that the receiver operating characteristic curves can be very different when different end points are used to describe abnormal pregnancy outcome. Thus the receiver operating characteristic curve for the prediction of fetal growth retardation is probably different from that used to predict operative delivery for fetal distress. This is demonstrated by the plots in Fig. 1, which are based on Gosling's pulsatility index data developed by Gudmundsson and Marsal.[1] It is conceivable that the discriminating ability of the various Doppler indices could be different for these different curves for different definitions of poor pregnancy outcome.

Perhaps, as Hanley[2] has suggested in a recent review, receiver operating characteristic curves could also be different depending on the types of patients and institutions in which the curve is developed and who the operators are. Thus Dr. Maulik may not have proved the general case that the resistance index will always be superior to the other Doppler indices.

Finally, I wish to thank Dr. Maulik for publicizing these new decision-making techniques. This method of analysis of receiver operating characteristic curves should be more widely applied to our diagnostic problems. They might help us decide whether competing tests, such as the nonstress test, contraction stress test, biophysical profile score, or other test of fetal wellness, or lack thereof, are to be preferred over the others. Indeed, receiver operating characteristic curve analysis may help us decide whether sequential testing schemes such as nonstress testing followed by contraction stress testing or biophysical profile scoring followed by contraction stress testing have any demonstrable advantage. Examples of similar applications have been introduced into our literature but appear to have received little recognition.[3, 4]

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Dr. William J. Ott, St. Louis, Missouri. This article is an extension of a study presented at this association in 1989.[1] As such, the study suffers from the same minor weakness of the previous study. Receiver or relative operating characteristic curves is a statistical method that has only recently begun to be used in obstetric research. This method was first used in medical analysis to evaluate radiologic instrumentation to determine the ability of various systems to differentiate between signal and noise. The test was designed to evaluate situations where a clearcut yes or no (positive or negative) result was expected. The extension of this test to situations where less clear-cut end-points, such as the clinical parameters used in this study, may make this a somewhat less accurate technique. But as mentioned in the critique of last year's study, there are no clear clinical criteria (other than mortality) that can be used to evaluate clinical outcome.

The results of today's paper indicate that the resistance index and systolic-to-diastolic ratio are slightly superior than other, more complicated mathematic methods of quantifying umbilical arterial Doppler waveforms. It is not too surprising that these two techniques have very similar accuracies, since they are almost identical, simplified mathematic methods of quantifying Doppler waveforms.

Since Doppler blood flow studies are becoming widely used as a method of fetal evaluation, the authors are to be congratulated for presenting one of the first detailed comparisons of the accuracy of the various Doppler indices currently in use.

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DR. CHESTER B. MARTIN, JR., Madison, Wisconsin. Since the same terms appear in the systolic/diastolic ratio and resistance index, I wonder if it is a mathematic fluke that the two curves appear different. For each value of systolic/diastolic, a corresponding resistance index can be calculated mathematically, and the reverse. It appeared to me that you had more points on your resistance index graph than on the systolic/diastolic curve. If you had evaluated the two indices at the corresponding values, would they not have given identical results?

DR. MAULIK (Closing). I would like to express my sincere thanks to Drs. Rightmire and Ott for their critique of our article. I also thank Dr. Martin and the colleague from Chicago for their interest in our work. I shall try to respond to their comments and questions in an adequate manner within the time limit.

The optimal threshold value of the resistance index for diagnosing adverse perinatal outcome in our population is 0.66. We determined this by receiver operating characteristic analysis in which the optimal balance between the true-positive and false-positive rates were determined by graphical assessment of the receiver operating characteristic curve derived from the measured operating points of the test value. We presented this information at the Second Congress of the International Perinatal Doppler Society in Paris in 1989. Regarding the comment on the ability of the resistance index to assess the negative end-diastolic flow, it is well recognized that this index reaches the value of unity when the end-diastolic frequency shift becomes zero, indicating an absence of forward flow. Obviously, it will not reflect any further changes in the frequency shift in the negative direction. Only the pulsatility index is capable of reflecting the latter phenomenon. However, the latter occurrence is very rarely encountered in the umbilical arterial circulation and represents further deterioration in the condition of a fetus in dire distress. Recently a modification of the resistance index has been described by Prof. Pourcelot; I have had the privilege to validate this along with Profs. Arbeille and Pourcelot. We shall have the opportunity to present this study at the next annual meeting of the American Institute of Ultrasound in Medicine.

Regarding the continuing usage of the systolic/diastolic threshold value of 3.0, the receiver operating characteristic analysis conducted by our group indicated a general optimal point of 2.9. As the measurement error may contribute up to 10% of the variance, there may not be any pragmatic difference between these two values. We presented these data during the last Central Association meeting. Regarding the relative efficacy of the various Doppler indices, a comparison of the areas under the receiver operating characteristic curve of these indices offers a more rational and convincing approach than that based on the arbitrary selection of a single operating point of a test. Dr. Rightmire is quite correct in emphasizing that the discriminatory ability of the various indices is very much dependent on the differing definitions of the outcome criteria. We certainly do not intend to give the impression that our demonstration of the superiority of the resistance index holds true for all circumstances and population types. Indeed, the diagnostic efficacy of Doppler velocimetry should be tested, taking into account various outcomes with differing prevalence rates, cost-benefit analysis, and the intended use of the test.

Regarding Dr. Martin's comment, we know that both the resistance index and the systolic/diastolic ratio are estimated from the same parameters, the peak systolic and end-diastolic frequency shifts, but they have different formulations. Because of its unique formulation, the systolic/diastolic ratio increases in an exponential manner with the progressive decline of the end-diastolic frequency shift, and it reaches the value of infinity with the absent end-diastolic flow. That is why this index not only becomes insensitive beyond a certain point, but also demonstrates maximum total variance of all the indices that we have measured. Thus, although one of these indices can be mathematically derived from the other, their diagnostic efficacies are not identical.

In relation to the outcome parameters, we have investigated the efficacy of the systolic/diastolic ratio in diagnosing intrauterine growth retardation. As we know, it is a multietiologic phenomenon, and, in our experience, the Doppler velocimetry probably does not offer the best method for diagnosing this condition.

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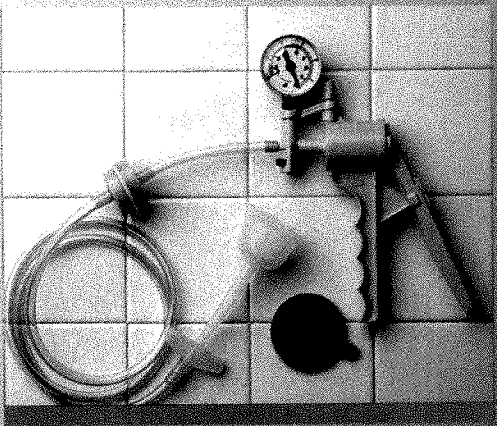

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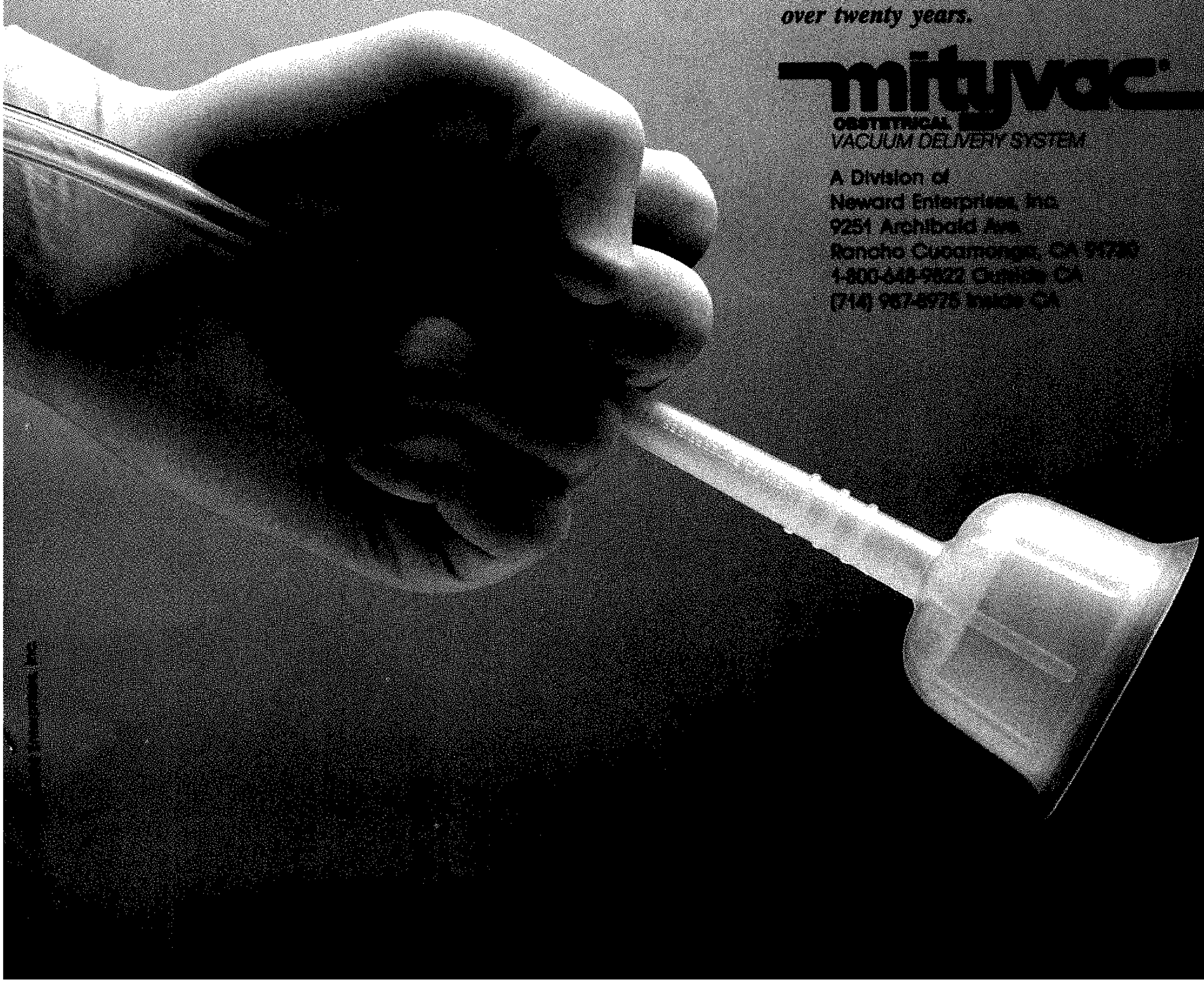

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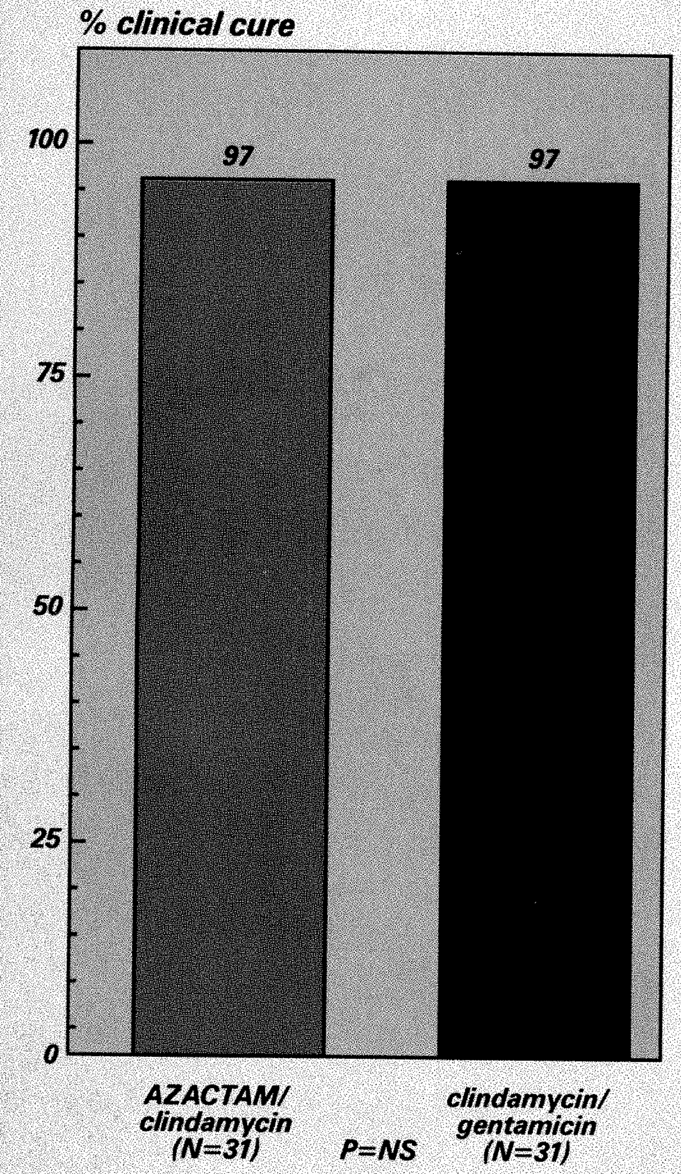

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[*] Due to susceptible organisms.

[†] Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

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INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

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*Efficacy for this organism in this organ system was studied in fewer than ten infections.

***References:* 1.** *Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* **2.** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* **3.** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

SQUIBB

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Vaginal birth after cesarean section: The impact of patient resistance to a trial of labor

Gerald F. Joseph, Jr., MD, Charles M. Stedman, MD, and Alfred G. Robichaux, MD
New Orleans, Louisiana

In spite of the relative safety and medical advantages of vaginal birth after cesarean section, the procedure continues to be underutilized in the private practice setting. To evaluate the hypothesis that resistance by the patient often precludes a trial of labor, an observational study was conducted of all women with a history of one prior cesarean section who were delivered in 1989 at Ochsner Foundation Hospital. The choices of 167 women and the judgments of their obstetricians were longitudinally recorded during the antepartum and intrapartum course. Patients routinely received the patient guide of the American College of Obstetricians and Gynecologists for vaginal birth after cesarean section. Ultimately, 50% of patients who were encouraged by their obstetrician toward vaginal birth after cesarean section opted for an elective repeat cesarean section without a trial of labor. Reasons for patient resistance are enumerated and potential future remedial proposals are discussed. (AM J OBSTET GYNECOL 1991;164: 1441-7.)

Key words: Vaginal birth after cesarean, repeat cesarean section, decision making

Vaginal birth after cesarean section (VBAC) rose to the forefront of obstetric controversy in the 1980s. Review of the literature suggests that the long-taught principle of "once a section, always a section" is no longer the best approach.[1,2] On Oct. 26, 1988, the American College of Obstetricians and Gynecologists (ACOG) updated their guidelines concerning vaginal delivery after previous cesarean section. The ACOG Committee on Obstetrics: Maternal and Fetal Medicine stated: "The concept of routine repeat cesarean birth should be replaced by a specific indication for a subsequent abdominal delivery and in the absence of a contraindication, a woman with one previous cesarean delivery with a low transverse incision should be counseled and encouraged to attempt labor in her current pregnancy."[3] This latest ACOG guideline endorses a concept of management that obligates the obstetrician to include the patient in planning the approach to delivery.

For 4 years the Department of Obstetrics and Gynecology at the Ochsner Clinic has had an active VBAC program. Ours are private patients and as such have retained an active role in matters pertaining to their health care. Even though we have encouraged all eligible patients to consider VBAC, some have refused. It is our perception that it is often the reluctance of the patient, not her obstetrician, that prevents greater utilization of VBAC. An interest in verifying or refuting this perception provided the incentive for this study.

Material and methods

The study population consisted of all patients with a history of one previous cesarean section who were delivered at the Ochsner Foundation Hospital between Jan. 1 and Dec. 31, 1989. All patients were managed throughout pregnancy by one of seven obstetricians at the Ochsner Clinic in New Orleans.

Patients were included in this study if they met the following criteria: (1) one previous low-transverse cesarean section, (2) singleton fetus, (3) cephalic fetal presentation, and (4) candidate for epidural anesthesia. Patients were excluded from the study for the following reasons: (1) medical complications of pregnancy necessitating immediate delivery or (2) ominous antepartum fetal heart rate testing that contraindicated a trial of labor.

Each patient was given a copy of the ACOG patient education pamphlet entitled "Vaginal Birth After Cesarean Delivery."[4] A report of the previous delivery was obtained, and specifics of the previous labor and the type of uterine scar were ascertained. On the basis of the obstetric history and pelvic examination early in pregnancy, the obstetrician recorded his/her initial preference as to mode of delivery. Each patient's initial choice as to delivery preference was likewise recorded after verbal discussion and review of the ACOG pamphlet. Changes in the patient's decision or the obstetrician's opinion were tracked longitudinally during the pregnancy along with specific explanations.

From the Department of Obstetrics and Gynecology, Ochsner Clinic and Alton Ochsner Medical Foundation.

Presented at the Fifty-eighth Annual Meeting of the Central Association of Obstetricians and Gynecologists, Louisville, Kentucky, October 11-13, 1990.

Reprint requests: Gerald F. Joseph, Jr., MD, Department of Obstetrics and Gynecology, Ochsner Clinic, 1514 Jefferson Highway, New Orleans, LA 70121.

6/6/28939

Table I. Patients excluded from study

Reason for exclusion	*No. of patients (n = 24)*
Vertical uterine incision	7
Abnormal presentation at term	8
Maternal medical complications	3
Abnormal antepartum testing	2
Multiple gestation	3
Lumbar disc disease precluding epidural anesthesia	1

Table II. Reasons for refusal of VBAC in group IV

Cited reason	*No. of patients (n = 41)*
Fear of labor	24
Convenience of scheduled delivery	11
Fear of recurrent outcome	6

Patients were considered "poor" VBAC candidates if, in the judgment of the obstetrician, they had a clinically contracted pelvis. All others were considered "good" VBAC candidates.

Oxytocin augmentation was freely utilized for the usual obstetric indications. Induction of labor was not offered routinely. Patients understood that epidural anesthesia was available on a 24-hour basis.

Results

A total of 167 patients with one previous cesarean section received their prenatal care and were delivered at the Ochsner Medical Institutions during the study period. This group represented 13% of the 1254 deliveries in 1989. Of these 167 patients, 24 (14.4%) were excluded from the study (Table I). For the purposes of this study 143 (85.6%) patients were considered VBAC candidates.

Of the 143 potential candidates, 12 (8.3%) were advised by their obstetricians early in the pregnancy that they were "poor" VBAC candidates (group I). None of these patients was encouraged by her obstetrician to attempt VBAC but by ACOG guidelines a trial of labor was not precluded. All of these patients elected a repeat cesarean section without a trial of labor. The weight of the newborns at the time of repeat cesarean section was greater than the weight at the time of the initial cesarean section in 9 of these 12 cases. The mean weight of the newborns at repeat section was 3500 gm as compared with 3256 gm at the primary cesarean section.

Group II consisted of patients judged to be "good" candidates for VBAC by their obstetricians and who consistently chose to attempt a vaginal delivery. Of the 143 patients, 46 (32.2%) were in this group and 30 of 46 (65.2%) achieved a vaginal delivery. Cephalopelvic disproportion was cited as the indication for a repeat cesarean section in 14 of the 16 patients who were unable to have a vaginal delivery. Patients 15 and 16 underwent repeat cesarean section because of fetal distress. The mean birth weight in the group delivered vaginally was 3210 gm (range 1240 to 4508 gm) whereas the mean weight in the group with unsuccessful trial of labor was 3543 gm (range 2700 to 4111 gm).

Group III consisted of 5 (3.5%) "good" candidates for VBAC who initially chose not to have a trial of labor. Three of these patients cited "fear of recurrent outcome" and two "convenience" as reasons for initially opting for repeat cesarean section. These patients subsequently abandoned their choice of elective repeat cesarean section when admitted in active labor between 12 and 24 days before the estimated date of confinement. All five were delivered vaginally.

Group IV consisted of 41 of 143 (28.7%) who were considered "good" candidates but who steadfastly refused a trial of labor. The primary reasons given by these patients for electing a repeat cesarean section without a trial of labor are seen in Table II.

Group V consisted of 39 of 143 (27.3%) "good" candidates who initially planned to attempt VBAC but who ultimately were delivered by repeat cesarean section without a trial of labor. Eleven patients (subgroup Va) initially chose VBAC in early pregnancy but later changed their minds despite continued encouragement by their obstetricians to pursue VBAC. All 11 cited "convenience" as their primary reason for change in plans.

Twenty-eight patients (subgroup Vb) initially chose VBAC in early pregnancy and were initially judged to be "good" candidates for a trial of labor but were advised by their obstetricians at term that there was a diminishing chance of vaginal delivery. Twenty-seven of 28 were considered to have an estimated fetal weight exceeding pelvic capacity. Ninety-three percent of these patients had had a primary cesarean section because of cephalopelvic disproportion. The mean birth weight at repeat cesarean section was 3886 gm.

The evolution of choices in "resistant" and "receptive" patients is illustrated in Figs. 1 and 2.

Comment

The medical literature in the past decade is replete with the low maternal and perinatal morbidity and mortality associated with VBAC.[5-11] Positional statements at the national level began in 1980 when the National Institutes of Health Consensus Conference on Cesarean Section convened in response to the sharply rising rate in cesarean births. On the basis of the available literature, this body suggested VBAC as one potential solution to the rising cesarean rate.[12] In 1982 ACOG issued their first guidelines relative to VBAC and deemed the procedure an acceptable option.[13] In 1985 VBAC guidelines were further expanded by ACOG.[14] The most recent guidelines, which were released in October 1988, cited VBAC as the preferred mode of delivery.[3]

Despite these reassuring data, VBAC has been slow to develop as a reality for many obstetricians. A hospital-based study in 1988 found only 12.6 VBAC deliveries per 100 women with previous cesarean sections.[15] Is this perpetuated utilization of repeat cesarean section in seven of eight cases a physician-driven or patient-driven phenomenon? Physician factors suggested as possible deterrents to a trial of labor have included failure to gain experience with VBAC during training years, the convenience of a scheduled delivery, fear of litigation, and even the issue of higher fees for abdominal delivery.

Surprisingly, there is a paucity of information regarding the attitudes and opinions of patients reluctant to agree to a trial of labor.[16, 17] Kirk et al.[16] sought to document reasons that patients chose repeat cesarean section by use of a retrospective questionnaire sent to postpartum patients. Six reasons were listed for choosing repeat cesarean section. The two major factors cited were avoidance of pain of labor and perceived low probability of vaginal delivery. Seventy-three percent of patients choosing repeat cesarean section reported their obstetricians either discouraged VBAC or had a neutral or unknown attitude. Unfortunately, statistical analysis was impaired by the 62% return rate. In our study we sought to eliminate many of the variables that might dissuade patients from a trial of labor.

The first national survey of ACOG members regarding attitudes and practices concerning VBAC was conducted in March 1990 and reported in late August 1990.[18] This survey found that approximately 60% of patients given the option chose VBAC. The survey may suffer from recall bias on the part of the responders, and therefore any conclusion drawn from these numbers must be suspect.

In our study group, 59% (groups II and V) *initially* chose to attempt a trial of labor (Fig. 1). However, *ultimately*, 64.3% (groups I, IV, and V) opted for an elective repeat cesarean section without a trial of labor (Fig. 2). Of the 85 patients who initially chose VBAC, 39 (group V) became disenchanted with the prospect of VBAC late in the pregnancy and opted for elective repeat cesarean section. By this time in the pregnancy, 28 (subgroup Vb) of these 39 patients were negatively influenced by their obstetricians' predictions of diminishing success if a course of VBAC was pursued. However, 11 (group Va) of the 39 opted for an elective repeat cesarean section despite continued enthusiasm and encouragement toward VBAC by their obstetricians. These 11 patients (subgroup Va) plus the 41 patients (group IV) who steadfastly opted for elective repeat cesarean section throughout pregnancy represent the total number of "resistant" patients in our study. In summary, 72% of candidates (groups II, III, and IV and subgroup Va) were given continued encouragement to pursue VBAC, yet only half of these opted for a trial of labor.

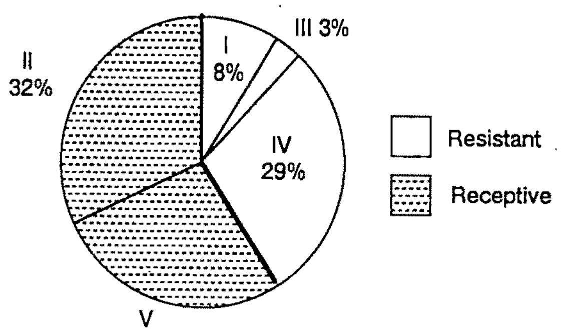

Fig. 1. Pie graph showing initial choice regarding trial of labor of each patient ($N = 143$) according to groups.

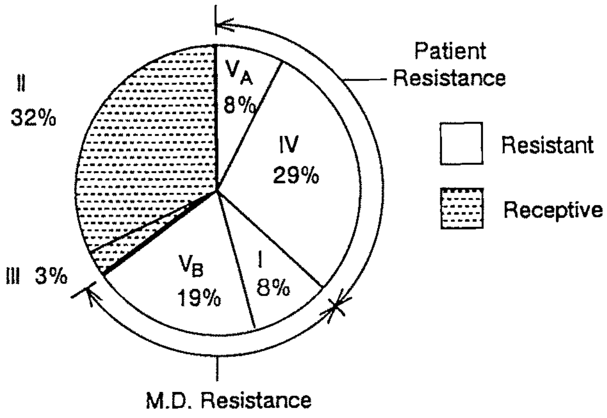

Fig. 2. Pie graph showing final choice regarding trial of labor of each patient ($N = 143$) according to groups. Patient-directed resistance and physician-directed resistance are shown.

Of the 51 patients with attempted VBAC (groups II and III), success was reported in 35 (68.6%), which is near the success rate quoted in other studies.[10, 19-21] In only five cases (group III) was the obstetrician able to change the patient's mind in favor of VBAC; in all of these cases the patient presented in active labor significantly before the estimated date of confinement.

Quantitating the influence of the obstetricians on the decisions made by their patients is most difficult. "Enthusiasm" for VBAC surely must vary depending on many interactions between the patient and her obstetrician.[16] By giving each patient an informational pamphlet, we provided an unbiased view of the subject for the patients' careful consideration. Certainly, the "negative" influence exerted by the obstetrician with regard to the 12 patients in group I and the 28 patients in subgroup Vb had a profound influence on their decision-making process. These 40 of 143 patients (28%) represent the "resistance" to VBAC by the obstetricians in our study. Undoubtedly, had all of the 40 patients been subjected to a trial of labor, some of these patients may have been delivered vaginally.[22-24] We remain convinced, however, that most of these patients would have labored unsuccessfully and feel supported in this belief by the birth weights.

Our study reveals that reluctance toward VBAC does not emanate exclusively from the obstetrician but also comes from some patients and that, despite efforts to provide a supportive setting, many patients still prefer operative delivery by repeat cesarean section rather than a trial of labor. These patients apparently view repeat cesarean delivery as an advantageous procedure that allows a known time of delivery without the uncertainties of attempted VBAC. Medical concerns pertaining to costs and safety seem to be overlooked by such patients.

Fear and inconvenience appear to be by far the most commonly cited deterrents to VBAC. One possibility is that some patients had already decided on repeat cesarean section even before conception. It may be that educational literature and physician counseling should be offered immediately after the primary cesarean section rather than held until a subsequent pregnancy as suggested by Kirk et al.[16] In our opinion enhanced patient education and neutralization of the convenience factor should theoretically reduce patient resistance to a trial of labor in the future. Perhaps this type of approach would be preferable to a protocol of mandated trial of labor.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Fredrik F. Broekhuizen, Milwaukee, Wisconsin. A recent report by the National Center for Health Statistics analyzing international differences in obstetric interventions indicated that in 1985 the United States had a 6.6% rate of women with a previous cesarean section who were delivered vaginally versus 3.4% in 1980.[1] It is likely that by now this percentage has further increased with continuing acceptance of VBAC and application of the ACOG guidelines regarding VBAC. I doubt that the national percentage, reaching 12.6% in a hospital-based study[2] in 1988, reached the 20.9% rate reported in this series in 1989. In several European countries, no significant changes in VBAC rates were seen between 1980 and 1985 with rates in 1985 ranging from 39.3% in Scotland to 55.4% in The Netherlands. Rates in these ranges might represent rather high targets, which are achievable at a national level, since they are reached in countries with medical practice climates where the management plans after a previous cesarean section are predominantly determined, if not mandated, by an obstetrician, and the patient is not likely to play as active a role in the decision-making process. Since the "once a section . . ." principle was never accepted in these countries mentioned, we can assume that the choice for a repeat cesarean section is not based on adherence to an outdated practice or resistance to a change in practice by obstetrician or patient. On the other hand, with lower primary cesarean section rates, a higher percentage of recurrent indications for repeat cesarean section would negatively influence the VBAC rates.

A 20.9% rate, given this background, after a 4-year VBAC program in a private-practice setting and with a nonmandated approach, is in my opinion not as disappointing as the authors indicate. It will require many more years before we will observe a final effect of the ACOG guidelines, which opened the door only in 1985 for a universally acceptable VBAC practice. In the largest reported VBAC series in the United States, 38% of patients with a previous cesarean section underwent a trial of labor with a 75% success rate and a vaginal delivery rate of 28.5% for all patients with a previous cesarean section (unpublished observations).

How unbiased or biased is counseling for a trial of labor? Is the ultimate summarizing message heard by the patient: "VBAC is safe, a recommended and optimal management for you, but you have the right to refuse this medically sound advice and elect a repeat cesarean?" Or is the message heard: "VBAC is safe, a reasonable and medically sound option, but truly, it's totally your choice?" I submit to you that we all have observed or even utilized both approaches, perceiving both as carrying the spirit of the ACOG guidelines, and that we assume that different VBAC rates will result. I feel the authors missed an opportunity to assess the patient's perception of the actual counseling message as an interfering factor for acceptance or rejection. Kirk et al.[3] concluded, as did the authors, that social exigencies appeared to play a more important role than assessment of medical risks in patient decisions. It is reported that physicians in public hospitals exert more influence on patient's decisions[3]—a subtle shift to mandate? In my own institution, with a distinct "we recommend, but" type of educational message, between 1985 and 1989 we saw the repeat cesarean section rate decrease from 9.5% to 5.0% and the VBAC rate rise from 2.1% to 5.5% of all deliveries. Of all patients with a previous cesarean section, 40.9% were delivered vaginally.

Would the authors address the following questions. First, how would they characterize the message sent by their group to their patients? Second, does their group's approach to VBAC deviate significantly from the approach taken by other obstetricians in their community and to what extent could that have influenced their patients' attitudes?

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DR. JAMES R. SCOTT, Salt Lake City, Utah. Because repeat cesarean section has contributed to the rising cesarean section rate, obstetricians and their patients have been strongly encouraged to attempt a trial of labor with subsequent pregnancies. The purpose of this study was specifically to investigate the incidence and reasons for patient refusal of VBAC. This is certainly a topic of current interest to all obstetricians since physicians often have been blamed for the slow acceptance of this concept. The prospective study design was satisfactory, but more information about the patients' ages, education, and socioeconomic status is needed to determine whether the results could be interpolated to other populations. It would also be helpful to provide more details on exactly *why* the patients refused a trial of labor. For example, were the women with a fear of labor or recurrent outcome those who had long, painful labors, poor outcomes, or complications associated with their labors that resulted in the previous cesarean section? In my experience these often have been factors. Moreover, an interesting group of patients that have received little attention in most publications on VBAC are those who again have failure of the trial of labor. These considerations are illustrated by two of my own patients who were delivered within 2 weeks of each other:

The first patient was delivered of a 4000 gm infant by cesarean section at 42 weeks' gestation in her first pregnancy after a long and difficult first stage of labor with a transverse arrest in the second stage. She did not want to go through that again, and I thought she again had a macrosomic infant. She underwent an uncomplicated repeat cesarean section, again was delivered of a 4000 gm infant at 39 weeks, was discharged in 3 days, and had a total hospital bill of $2,408.

The second patient's first cesarean section was because of a breech presentation, and she wanted a postpartum tubal sterilization. She also was not enthusiastic about VBAC, but I convinced her to try it. This time she had a long labor with many vaginal examinations. She pushed for >2 hours in the second stage, but the presenting vertex remained high. She was again eventually delivered by cesarean section, and a tubal ligation was done. The baby did well, but the patient was febrile and required antibiotics. A wound infection developed and she was in the hospital 9 days with a total cost of hospitalization of $5,806.

In other words, the patient that I persuaded to attempt a trial of labor failed after about 24 hours of labor, was in the hospital three times as long, and had a hospital bill more than twice as high as that of the patient who had a repeat cesarean section. Which patient do you think was happiest with her management?

Do the authors have any information on the patient satisfaction and complication rates of those patients who failed a trial of labor as compared with those who simply underwent repeat cesarean section or had a successful VBAC?

Although VBAC appears to be a safe and desirable option, it should be pointed out that none of the studies reported in the literature have been prospective trials randomizing repeat cesarean section with a trial of labor to actually prove that maternal and neonatal outcomes are better with the latter. In our own metropolitan area, since 1982 there have been 12 known uterine ruptures with attempted VBAC, which have resulted in three perinatal deaths and two severely damaged infants; in four of these cases litigation has occurred or is in progress.

Finally, I believe that the most interesting question this study raises is just how strongly the physician should encourage, persuade, or even coerce a patient to attempt a trial of labor if she prefers a repeat cesarean section. In my mind this question has never been satisfactorily addressed either medically or legally. Originally, the National Institutes of Health consensus group recommended offering VBAC to selected patients; ACOG guidelines later stated that VBAC should be encouraged for all patients, and others have implied that the patient should be given no choice. This has apparently now led some third-party payers to require a trial of labor.

Therefore what did the authors learn from their study that will be helpful to all of us confronted with the present situation? Do they recommend that we continue to encourage patients to participate in the decision regarding the route of delivery even if some refuse VBAC? On the other hand, if they believe that patient acceptance of VBAC should be increased, exactly what "enhanced patient education" and "neutralization of the convenience factor" do they recommend, and what is the evidence that these measures will be effective?

I congratulate the authors on their timely study.

Dr. Washington Hill, Nashville, Tennessee. The authors do show that resistance for VBAC does not emanate completely from the obstetrician. They also show that the obstetricians play a very important part.

I would like to draw attention to two groups of the authors—group I and group Vb. What is meant by "clinically abnormal pelvis" and what is meant by "larger than pelvic capacity at term"? Those two terms are one of the reasons why physician resistance occurs.

Dr. Leland R. Matthews, Bloomington, Indiana. I applaud your study of this important clinical problem. I think in our practice the resistance of the patient with prior cesarean section to again experience labor is the single greatest obstacle to VBAC. However, I have a question. Why does a VBAC candidate also have to be a candidate for epidural anesthesia?

Dr. Peter F. Fehr, St. Paul, Minnesota. I was going to ask the same question. In the review of our VBAC rate, we've discovered that our resident clinic, which has an expectation of VBAC, has the best VBAC rate in our institution whereas some of our obstetricians have a zero VBAC rate. In fact, some of the patients are moving to other obstetricians who are willing to do VBACs.

My question is was there a difference between physicians? The other question I ask is what is the approach at the time of the primary cesarean section? We discovered that many of the patients that come into our program have been told essentially nothing or basically have been told that they should not consider a VBAC at the time of their first cesarean section.

Those who have originally said, well, you can probably plan a vaginal delivery the next time, come into their next pregnancy with a totally different attitude. I think the way you end up with your first cesarean section is to scare the patient. You actually prepare her for a VBAC or a non-VBAC for the next delivery.

Secretary-Treasurer Anderson. Was there any impact of third-party payers demanding VBAC consideration? Did this influence your study?

Dr. Joseph (Closing). I thank Dr. Broekhuizen and Dr. Scott for their discussion and I also appreciate the other interest. I think that VBAC and repeat cesarean section is a difficult topic. It's a topic that interested us enough to spend 1 year tracking our results. Basically we did so because we just wanted to see what we were doing.

Dr. Scott asked what we learned from the study. I think we learned that perhaps the best way to handle VBAC is to continue to be good clinicians.

One of the floor questions was what we considered a small pelvis. Basically this was not an x-ray diagnosis but a clinical diagnosis made by the experienced obstetrician. The "big" fetus was "big" mostly by ultrasonography, but it was a *relative* "big." The fetus didn't necessarily have to weigh 4200 or 4000 or 3800 gm. It was relatively big for the mother's pelvis; again, these were clinical decisions.

Dr. Broekhuizen asked what did we think our counseling message was. Everybody's counseling message is a little different. Our counseling message was "We recommend," much like his. I think if patients are given the ACOG pamphlet and physicians talk to them about their chances of vaginal delivery and about their complications, patients elect different things. Some patients may elect a primary cesarean section because of the fear of herpes, even if they've had herpes once in their life, and sometimes you can't talk them out of that.

Dr. Matthews asked about epidural anesthesia. What we tried to do was to put in this group only those patients who could not lay blame on not wanting a VBAC on anything else. They knew they had the availability of anesthesia, so they couldn't say, "I don't want to do VBAC because you don't have anesthesia." It's not that all patients had epidural anesthesia, although most of our patients do.

I doubt very seriously that our VBAC rate is totally different from others in the community. We do have a teaching program at our hospital, so maybe we are a little more liberal in our policies for VBAC than other institutions, but by no means would I consider our institution to be known for its VBAC program.

Dr. Anderson asked about third-party payers. Third-party payers were tracked in this study but not reported here. A large number of our patients are health maintenance organization patients; interestingly enough, their outcome was no different than the outcome for private patients.

Again, in conclusion, I would say that one of the things we learned from this study is that perhaps the time of the primary cesarean section is the time that counseling for the next delivery should start. Patients are often discouraged at this time, and they look down the road to see more cesarean sections, and it's certainly at that time that the ground should be laid for the method of delivery in future pregnancies.

Pregnancy rates after peritoneal ovum-sperm transfer

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Indianapolis, Indiana

We present the technique of peritoneal ovum-sperm transfer as an option for treatment in couples with unexplained infertility factors. In 1989 we reported the first successful pregnancy, in the United States, after transferring sperm and oocyte into the peritoneal cavity. We now report the results of a prospective study of this procedure. Twelve women with unexplained infertility underwent 23 cycles of peritoneal ovum-sperm transfer. Ovulation stimulation was achieved with human menopausal gonadotropin. Ultrasonographically directed oocyte recovery was performed by the transvaginal route with the patient under local anesthesia and sedation. After oocyte recovery, 4.5 ± 0.4 (mean ± SE) oocytes and 13.3 ± 1.0 (mean ± SE) × 10^6 motile spermatozoa were transferred into the pouch of Douglas. Six clinical pregnancies occurred in 23 stimulated cycles for a pregnancy rate of 26% per cycle. This value compares with the overall pregnancy rates of 16% for in vitro fertilization and 27% for gamete intrafallopian transfer reported by the In Vitro Fertilization Registry. Thus these preliminary data suggest that peritoneal ovum-sperm transfer is at least as successful as in vitro fertilization and gamete intrafallopian transfer. Advantages of peritoneal ovum-sperm transfer over gamete intrafallopian transfer include its being an office nonsurgical procedure not necessitating a general anesthetic and decreased cost. Therefore peritoneal ovum-sperm transfer is a reasonable first approach in couples with unexplained infertility. (Am J Obstet Gynecol 1991;164:1447-52.)

Key words: Peritoneal ovum-sperm transfer

Infertility is a significant public health concern affecting an estimated 3 million couples in the United States.[1] Despite complete investigation, the cause of infertility is unexplained in approximately 20% of couples.[2] For treatment of these individuals, assisted reproductive techniques have become increasingly more common over the last decade. The procedure associated with the highest pregnancy rate reported to the In Vitro Fertilization Registry[3] is gamete intrafallopian transfer. Pregnancy rates after this procedure are reported to be 27% per cycle in which gametes were transferred.[3] However, to achieve these results an operative procedure and a general anesthetic are required. Pregnancies after transfer of oocytes and sperm into the peritoneal cavity with local anesthesia and sedation in an

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6/6/28225

Table I. Clinical characteristics of peritoneal ovum-sperm transfer cycles

	No.	*%*
Patients	12	
Cycles	24	
Cancelled	1	4
Transferred	23	96
Pregnancies	6	26

Table II. Summary of gamete characteristics of peritoneal ovum-sperm transfer cycles

	Mean ± SE
Transfer cycles	23
Follicles aspirated (mean ± SE)	15.9 ± 2.0
Oocytes recovered (mean ± SE)	7.6 ± 1.2
Oocytes transferred (mean ± SE)	4.5 ± 0.4
Motile sperm transferred (mean ± SE) × 10^6	13.3 ± 1.0

Table III. Pregnancy rates resulting from peritoneal ovum sperm transfer procedures

Study	*No. of patients*	*No. of cycles*	*Pregnancies*				
			Total		*Abortions*		
			No.	*%*	*No.*	*%*	*Ectopic*
Mason et al.[4]	9	9	4	44	1	25	0
This series	12	23	6	26	2	33	0
TOTAL	21	32	10	31	3	30	0

office setting have been documented.[4-6] We have previously reported the first pregnancy resulting from peritoneal ovum-sperm transfer in the United States[4] and the lack of production of sperm antibodies in women undergoing intraperitoneal insemination of sperm.[6] We now report results of a prospective study of this procedure by the transvaginal route under ultrasonographic guidance and local anesthesia. Six clinical pregnancies occurred in 12 women undergoing 23 stimulated cycles for a pregnancy rate of 26% per cycle. It is concluded that peritoneal ovum-sperm transfer is as efficacious as gamete intrafallopian transfer in producing pregnancies in couples with unexplained infertility and that it is associated with lower risk and less cost than gamete intrafallopian transfer. Therefore peritoneal ovum-sperm transfer should be considered among the options as a first approach in treatment of couples experiencing unexplained infertility.

Material and methods

Patient population. Twelve couples with the diagnosis of unexplained infertility served as the study population. All of the women had the following normal or negative test results: ovulation documented by urinary luteinizing hormone testing and ultrasonographic monitoring of folliculogenesis[7]; luteal phase serum concentrations of progesterone; in-phase endometrial biopsy specimen; serum levels of thyroid-stimulating hormone, thyroxine, and prolactin; hysterosalpingogram; laparoscopic documentation of bilateral tubal patency, absence of pelvic adhesions, and absence of endometriosis; and negative sperm antibody assays. All of the men had normal semen analyses, normal penetration rates within the hamster egg penetration assay, and negative sperm antibodies. The average age of the women was 30 (range, 26 to 39) years and the mean duration of infertility was 3.9 (range, 2 to 6) years.

Ovarian stimulation and oocyte retrieval. Ovarian stimulation was achieved with intramuscular administration of human menopausal gonadotropin (150 IU/day) beginning on day 3 of the menstrual cycle. The serum estradiol level was monitored daily, typically between days 7 and 12. Luteinizing hormone was measured from urine collected every 8 hours on days 7 through 12. The endocrine parameters, in combination with ultrasonographic data, were used to determine the timing of exogenous administration of human chorionic gonadotropin (hCG) (10,000 IU) and subsequent oocyte retrieval, which occurred 36 hours after human chorionic gonadotropin administration. Follicular aspiration was accomplished by transvaginal, ultrasonographically guided needle aspiration, as previously reported.[5] Follicular contents were collected in Ham's F-10 medium (Gibco, Grand Island, N.Y.) supplemented with 10 mmol/L HEPES and 100 IU/ml heparin. Recovered oocytes were transferred into Ham's F-10 medium supplemented with 15% heat-inactivated fetal cord serum until retransferred to the cul-de-sac.

Sperm preparation. Sperm preparation was performed as previously described.[8] Briefly, the sample was allowed to liquify at room temperature for 30 minutes and then washed twice in Ham's F-10 medium containing 7.5% heat-inactivated fetal cord serum. The sperm pellet was overlaid with 1 ml of Ham's F-10 medium and incubated for 90 minutes at 37° C in a 5% carbon dioxide high-humidity incubator. The supernatant was removed and evaluated for sperm concentration and percent motility.

Gamete transfer procedure. Mature oocytes with approximately 13.3 ± 1.0 (mean ± SE) million motile sperm were transferred in Ham's F-10 medium supplemented with 15% heat-inactivated fetal cord serum into the cul-de-sac under ultrasonographic guidance by a 17-gauge spinal needle and a 3 Fr. Teflon catheter.

Luteal phase support and monitoring. Each woman received vaginal progesterone suppositories (25 mg/day), beginning 48 hours after the procedure for 14 days or until the onset of menses or as long as serum hCG levels were ≥20 mIU/ml. Blood was drawn for hCG determination beginning 10 days after peritoneal ovum-sperm transfer and repeated every 48 hours until onset of menses or demonstration of an intrauterine gestational sac with a 7.5 mHz vaginal ultrasonographic transducer. If hCG was ≥10 mIU/ml, transvaginal ultrasonographic examinations were performed beginning 14 days after peritoneal ovum-sperm transfer and were continued at weekly intervals until fetal heart activity was demonstrated.

Results

Table I summarizes the clinical characteristics of the peritoneal ovum-sperm transfer cycle. Table II describes the gamete characteristics of 23 peritoneal ovum-sperm transfer cycles. After oocyte recovery, 4.5 ± 0.4 oocytes and $13.3 \pm 1.0 \times 10^6$ motile spermatozoa were transferred into the pouch of Douglas. Six clinical pregnancies (26% per cycle) occurred. Three pregnancies terminated in spontaneous abortion before 8 weeks' gestation. One of these embryos had a chromosome analysis revealing 47,XX + 22 and another had 47,XX + 15. Three pregnancies produced six healthy infants (one singleton, one twin, and one triplet gestation). The singleton and twin gestations were delivered at term without complications. The triplets were born at 32 weeks' gestation and weighed between 1107 and 1532 gm. All infants had normal examinations at 1 year of age.

Comment

Assisted reproductive technologies continue to be increasingly important treatments for infertility. They are particularly helpful in treating individuals who suffer from infertility as a result of altered or prevented sperm-egg interaction. Since identification of such subpopulations is limited by available diagnostic tools, most of these couples are first seen for unexplained infertility.

We have previously reported a pregnancy resulting from a transvaginal, ultrasonographically guided peritoneal ovum and sperm transfer.[5] The results of this prospective study suggest that this procedure produces pregnancy in 26% of transfer cycles in couples with unexplained infertility. Pregnancies also have been reported after transabdominal, ultrasonographically guided peritoneal transfer of sperm and oocytes[4] at a rate of 44% per transfer cycle. Pregnancy rates resulting from peritoneal ovum-sperm transfer procedures are summarized in Table III. Overall, 31% of cycles in which gametes are transferred result in clinical pregnancies. No ectopic pregnancies have been reported. The pregnancy rate of 31% per cycle transferred by peritoneal ovum-sperm transfer compares favorably with the pregnancy rate of 27% per cycle transferred reported by the In Vitro Fertilization Registry for gamete intrafallopian transfer.[3] The success rates have led to wide acceptance of gamete intrafallopian transfer. While these initial data suggest that peritoneal ovum-sperm transfer has the same success rate, it has a number of advantages over gamete intrafallopian transfer. Gamete intrafallopian transfer requires a laparoscopy or minilaparotomy, and it is costly and potentially hazardous. Peritoneal ovum-sperm transfer is performed in the office with the patient under local anesthesia and intravenous sedation. The procedure is short in duration (<1 hour) and does not require hospitalization, general anesthesia, or an operative procedure. The cost is considerably less than for gamete intrafallopian transfer (about half). Therefore peritoneal ovum-sperm transfer offers a simpler and safer method for those couples undergoing assisted reproductive techniques for the treatment of unexplained infertility.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. George M. Grunert, Houston, Texas. To be accepted, a new procedure or treatment must meet one of several criteria: It must be safer, cheaper, or more efficacious or provide more diagnostic information than established methods. I will examine peritoneal

Table I. Superovulation and intrauterine insemination for unexplained infertility

	No. of cycles	*Pregnancies*
Dodson et al.[1]	38	8 (21.1%)
Yovich and Matson[2]	479	56 (11.7%)
Horvath et al.[3]	276	36 (13.0%)
Serhal et al.[4]	34	9 (26.4%)
TOTAL	827	109 (13.2%)

Table II. Zygote intrafallopian transfer as a successful treatment for unexplained infertility

No. of zygotes	*No. of cycles*	*Pregnancies*
1	3	0 (0%)
2	9	5 (55%)
3	42	21 (50%)
TOTAL	54	26 (48%)

Modified from Devroey et al.[5]

ovum-sperm transfer in this light, comparing it with superovulation and insemination, gamete intrafallopian transfer, in vitro fertilization, and zygote intrafallopian transfer.

Peritoneal ovum-sperm transfer is less expensive than gamete or zygote intrafallopian transfer, which require laparoscopy or laparotomy for transfer. In my institution, peritoneal ovum-sperm transfer would be marginally less expensive than in vitro fertilization with uterine transfer, and more expensive than superovulation and insemination. Similarly, it is safer than gamete or zygote intrafallopian transfer, equivalent to in vitro fertilization, and riskier than superovulation and insemination, assuming an equivalent risk of hyperstimulation syndrome.

Efficacy is difficult to compare. In their definition of unexplained infertility, the authors have omitted evaluation of cervical mucus, sperm transport, and immunologic factors. Since other authors exclude couples with such abnormalities from the definition of unexplained infertility, across-the-board comparison with other series is unfair. Table I presents data from other publications on the pregnancy rate from superovulation and insemination in couples with unexplained, cervical, or immunologic infertility. These series are heterogeneous and retrospective but demonstrate an overall pregnancy rate of 13.2% per cycle, not significantly different from that reported for peritoneal ovum-sperm transfer ($p > 0.05$). Peritoneal ovum-sperm transfer should have a marginal advantage over superovulation and insemination because the number and quality of oocytes presented to the tubes can be controlled.

A direct comparison with large gamete intrafallopian transfer or in vitro fertilization series (as presented by the authors) is not applicable because these procedures are reported without respect for their indications. Devroey et al.[5] reported on zygote intrafallopian transfer in patients with unexplained infertility (Table II). The pregnancy rate of 48% per cycle is not significantly greater than that reported for peritoneal ovum-sperm transfer. The ongoing pregnancy rate of 37% (20/54) is significantly greater than the 13% rate for peritoneal ovum-sperm transfer ($p < 0.05$). Zygote intrafallopian transfer also confirms oocyte fertilization, an important concern in patients with unexplained infertility. The future possibility of zygote transfer by transcervical cannulation may obviate the need for laparoscopy in these patients.

Could superovulation and insemination supplant peritoneal ovum-sperm transfer? The 342 couples undergoing gamete intrafallopian transfer at Woman's Hospital of Texas could be considered to have unexplained infertility or failure to conceive despite treatment of nonmale factor infertility. In 63 couples who had undergone three superovulation and insemination cycles without conception before doing gamete intrafallopian transfer, there were eight pregnancies (12.7%). In 279 couples who had not undergone superovulation and insemination, there were 75 pregnancies (26.7%) ($p < 0.05$). For this reason, I have recommended that couples in this category who failed to conceive after superovulation and insemination consider zygote intrafallopian transfer. I do not feel that gamete intrafallopian transfer is appropriate for these couples. Because the rationale is similar, I do not feel such couples would be good candidates for peritoneal ovum-sperm transfer.

I have several questions for the authors. What was the fate of supernumerary oocytes obtained? If they were fertilized at in vitro fertilization, was there any correlation between the fate of these sibling oocytes and pregnancy at peritoneal ovum-sperm transfer? In view of the increasing consumer concern about veracity of reporting and the 50% rate of spontaneous abortion in this series, would it not be better to emphasize viable or "take-home" pregnancy rates rather than overall clinical pregnancy rates?

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Dr. Pon Jola Coney, Tucson, Arizona. In vitro fertilization and embryo transfer were developed to treat infertile patients with damaged or absent fallopian tubes. The technique also has been shown to be effective in patients with non–tubal factor infertility. In an effort to improve the results in treatment, other innovative procedures of assisted reproduction have been developed.

Gamete intrafallopian transfer is based on the assumption that transfer of oocytes and spermatozoa to the fallopian tube would provide the most optimal conditions for fertilization and early embryo growth, because this is the natural site for these early events.

Direct intraperitoneal sperm insemination is based on the assumption that sperm transport along the fallopian tube is abnormal.

Peritoneal ovum-sperm transfer is based on the assumption that oocyte release does not occur or that sperm transport also is defective.

There are now reports consisting of approximately 50 patients who have undergone direct intraperitoneal sperm insemination with a pregnancy rate of 25%. Including the present report, there are now approximately 21 patients who have undergone peritoneal ovum-sperm transfer with a pregnancy rate of 26%. As reported by the In Vitro Fertilization Registry, the pregnancy rate for gamete intrafallopian transfer has consistently remained 27%.

The problem with both direct intraperitoneal sperm insemination and peritoneal ovum-sperm transfer lies within the small number of patients treated and the lack of larger, prospective, randomized, controlled studies comparing gamete intrafallopian transfer, direct intraperitoneal sperm insemination, and peritoneal ovum-sperm transfer in patients with non–tubal factor infertility.

If the techniques are equally successful, direct intraperitoneal sperm insemination would offer advantages over peritoneal ovum-sperm transfer and gamete intrafallopian transfer, with the latter two procedures being reserved for patients with failure of direct intraperitoneal sperm insemination or who would receive gamete intrafallopian transfer as an incidental procedure when laparoscopy is indicated.

Potential problems with peritoneal ovum-sperm transfer

Heterotopic pregnancy. Heterotopic pregnancy (concomitant intrauterine and extrauterine pregnancy) experienced an incidence of 1 in 25,000 pregnancies until the number of superovulation and assisted reproductive procedures increased. Most recently, several reports demonstrate an increase in heterotopic pregnancy in patients undergoing superovulation, in vitro fertilization, and gamete intrafallopian transfer procedures to an incidence of 1 in 200. Is this a result of spontaneous fertilization of unrecovered oocytes? Is there embryo migration from uterus to tube or from tube to peritoneal cavity, and would this incidence further increase if we directly placed sperm and ova in the peritoneal cavity?

Antisperm antibodies. The authors have recently reported a lack of antisperm antibody production in 11 patients undergoing intraperitoneal insemination of sperm and ova. Livi et al. have reported on 58 patients who were treated with at least two cycles each of direct intraperitoneal sperm insemination; in 28% antisperm antibodies had developed. In approximately half of these patients the antibody detection was transient in nature. With these contradictory reports the question is not resolved as to whether intraperitoneal inoculation of washed spermatozoa is very likely to result in massive antigen stimulation activating immunity to spermatozoa.

Finally, the benefit of peritoneal ovum-sperm transport, as in direct intraperitoneal sperm insemination and gamete intrafallopian transfer, to this particular group of infertile patients remains to be established. Infertility evaluation, age distribution, response to stimulation, indications for treatment, and durationof infertility all influence the prognosis for pregnancy, notwithstanding semen quality. Treatment-independent infertility, particularly in unexplained infertility, has long been known to result in spontaneous pregnancy rates in the range of 30%.

In conclusion, this is a very interesting report that could have dramatic influence on the clinical management of non–tubal factor infertility and clearly merits further clinical study.

Dr. Antonio Scommegna, Chicago, Illinois. When new techniques are investigated, it is important to compare them with more established techniques. The technique reported was compared with the results obtained with in vitro fertilization and gamete intrafallopian transfer procedures as reported by the National In Vitro Fertilization Registry. Unfortunately, the Registry is a compendium of all registered pregnancies—rates are not clinic specific and such rates may vary from 0% to 40%.

In our institution, for instance, in vitro fertilization and gamete intrafallopian transfer give essentially similar results except for male factors. Therefore in most instances in vitro fertilization is preferable. My question is, what are the specific rates for in vitro fertilization and gamete intrafallopian transfer in Dr. Coulam's program?

Also, what are the multiple pregnancy rates in this study? Since most programs now use gonadotropin-releasing hormone down regulation of the pituitary, I wonder if Dr. Coulam has, or if she is going to have, a series where down regulation is used before stimulation. The results may improve significantly.

Dr. Coulam (Closing). I thank Dr. Coney, Dr. Grunert, and Dr. Scommegna for their kind comments. I'll answer them as follows.

Dr. Grunert pointed out a comparison of the peritoneal ovum-sperm transfer procedure results with the other procedures available, and I have summarized these for you. These are a compilation of the world's literature reports of these procedures in a 3 × 3 matrix. There are sperm, egg, and fertilized egg; you can put those things in three different places—the uterus, the tube, and the peritoneal cavity. As pointed out, the pregnancy rates go up as you go in this direction.

There doesn't seem to be much benefit between these in terms of statistical results. The advantage, of course, is that this is an office procedure that takes 30 minutes, with half the cost, and it lacks the risk; this compares with the 13% result just for the superovulation intrauterine insemination.

Dr. Grunert asked about the fate of the extra eggs, and there are two possible fates. One, the patient is given the option of combining peritoneal ovum-sperm transfer with in vitro fertilization, but many times this is not a good economic option for them because they chose peritoneal ovum-sperm transfer. In this particular instance, they have a second option of donating eggs to individuals who don't have eggs.

Dr. Coney asked about sperm antibodies. We had careful follow-up of all these patients at monthly intervals, and we are mostly convinced that we are not inducing the formation of circulating antibodies to sperm in those women.

Dr. Scommegna very appropriately asked how these results compare with the results in our own institution, and just as in his program, we don't do many gamete intrafallopian transfer procedures because of the necessity of a general anesthetic and an operative procedure. So I don't have the results to share with you, but I do have our in vitro fertilization results.

In the particular year that we collected these 13 peritoneal ovum-sperm transfers, we had a 17% pregnancy rate per cycle transferred, which is analogous to the Registry results.

Our multiple pregnancy rate is high. It is about 60% in the peritoneal ovum-sperm transfer procedure, and, yes, we do use gonadotropin-releasing hormone for our standard in vitro fertilization ovulation induction at this time. At the time the peritoneal ovum-sperm transfer study was begun, we were not using gonadotropin-releasing hormone routinely.

President Malinak. Dr. Coulam, what about the abortion rate?

Dr. Coulam. We had six pregnancies with six babies and three abortions. Two of those abortions were clinical abortions and one was subclinical.

Oral contraceptives and antithrombin III: Variations by dosage and ABO blood group

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Detroit, Michigan, and Baltimore, Maryland

A randomized clinical trial of oral contraceptives evaluated 67 women on a regimen of 50 μg ethinyl estradiol and 1.0 mg norethindrone, 61 women on a regimen of 35 μg ethinyl estradiol and 1.0 mg norethindrone, and 64 women on a regimen of 35 μg ethinyl estradiol and 0.5 mg norethindrone. ABO blood group type was determined in all women. At baseline and at 3, 6, and 9 months, plasma antithrombin III levels, by both an immunologic and an activity method, and selected plasma levels of the contraceptive steroids were measured. Antithrombin III levels for all oral contraceptive groups combined decreased from baseline by 19.7% and 28.8% for the immunologic and activity methods, respectively. Analysis of interrelationships among antithrombin III by activity method, oral contraceptive type, and ABO blood group showed larger declines in antithrombin III for type O women using the highest estrogen dose preparation (31.6%) and for non–type O women using the lowest progestin dose preparation (38.9%). Plasma levels of contraceptive steroids also were related to changes in the most extreme levels of antithrombin III. (AM J OBSTET GYNECOL 1991;164:1453-60.)

Key words: Oral contraceptives, antithrombin III, ABO blood group

A number of studies have established a relationship between oral contraceptive use and changes in antithrombin III levels.[1-7] In addition, several studies have examined changes in antithrombin III levels and their relationship to thromboembolism.[8-12] Most investigations have suggested that the estrogen component in oral contraceptives is responsible for changes in antithrombin III, although this point has not been fully evaluated, particularly with current low-dose oral contraceptives.[11, 13] Further, there have been no randomized clinical trials that have examined differences among oral contraceptive preparations relative to antithrombin III, nor have there been any studies that have examined dose relationships in any detail. Finally, analyses have been confined to examining mean changes, although earlier studies by our group suggest the "outlier" analyses (study of subjects at the extremes) may add useful information.[14]

The current study is part of a series of clinical experiments designed to examine the relationship between oral contraceptive use, changes over time of selected lipids and lipoproteins, and blood pressure, as well as antithrombin III, and various measurements of contraceptive steroid levels as determined in plasma. Separate reports have addressed changes in lipid and lipoprotein levels and the relationship of some of these changes to contraceptive steroid levels.[14, 15] Analysis of patterns of plasma levels of contraceptive steroids, using 24-hour curves, indicates that steroid levels 24 hours after ingestion of the last oral contraceptive tablet and the initial slope or change in plasma levels after ingestion of a subsequent tablet provide an estimate of the metabolic behavior of these steroids.[1, 4]

This article presents additional results of a randomized clinical trial of healthy, young women using three different types of oral contraceptives relative to changes in antithrombin III levels, determined immunologically and with an activity test, during a 9-month period. In addition, information is presented on the relationship between certain metabolic measurements of the contraceptive steroids and the changes found in antithrombin III. The role of ABO blood type, a potential risk factor for thromboembolism, was also examined in relation to oral contraceptive use and levels of antithrombin III.[16, 17] The data also were analyzed with an approach to identify subjects in an outlier status according to the specific antithrombin III measurement.

Material and methods

Previous publications by our group have outlined in detail the basic study design.[14, 15] At the patient's initial visit, a complete blood count, ABO blood typing, and

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complete urinalysis were obtained. For the antithrombin III determination, after an overnight fast, venous blood was drawn into tubes containing 3.8% trisodium citrate. After immediate transport to the Johns Hopkins Coagulation Laboratory, plasma was prepared by centrifugation of the anticoagulated blood for 15 minutes at 3500 rpm at 4° C. Assays were performed within minutes after plasma preparation; all specimens were measured in duplicate. Two approaches were used to determine antithrombin III levels: an immunologic test and a test of activity level. Results were expressed as percent activity compared with similar determinations in a pool of 200 healthy blood donors. Subjects were sampled on at least two occasions, 48 hours apart.

The immunologic test involved assaying antithrombin III for its presence as an immunologic protein. Antithrombin III antigen as an immunologic protein was quantified according to the technique described by Weeks[18] and Laurell,[19] using monospecific antisera obtained from Behring Diagnostics (La Jolla). Normal range with this technique is 71% to 200% of that obtained in normal subjects. For the activity test, antithrombin III was quantified as a biologically functional protein by measuring its inhibitory effect on activated factor X (Xa), by means of a previously described technique.[20-22] The normal range for this approach is 55% to 100%.

Subjects were separated into one of the three oral contraceptive groups by computer-generated, restricted randomization at the time of the second set of baseline antithrombin III determinations. Cards identifying the assignment to one of the three oral contraceptive groups were placed into opaque envelopes. The oral contraceptives used in this study are commercially available and included: ethinyl estradiol 50 μg plus norethindrone 1 mg, ethinyl estradiol 35 μg plus norethindrone 1 mg, and ethinyl estradiol, 35 μg plus norethindrone 0.5 mg. At the time of the second antithrombin III determination, after blood was first obtained in the trisodium citrate tubes, about 15 ml of venous blood was placed into heparinized tubes and centrifuged to obtain plasma; levels of ethinyl estradiol and norethindrone were determined as described previously.[14] One hour after ingestion of the first oral contraceptive pill, levels of these contraceptive steroids were again determined.

During the third, sixth, and ninth oral contraceptive cycles, we again obtained venous blood for antithrombin III determination after an overnight fast. In addition, samples for plasma steroid levels also were obtained. Sampling was done on day 21 of the pill cycle, although there were rare exceptions to this rule because of logistic considerations. In addition, follow-up questionnnaires and abbreviated physical examinations, including blood pressure determinations as described, were completed at each visit.

Outlier status was defined separately for both measurements of antithrombin III. We arbitrarily chose baseline values below the 10th percentile for women in this study to define outlier status. The cutoff points determined from the baseline data also were used to define outlier status at the subsequent study intervals.

The 0-hour contraceptive steroid level corresponded to the level obtained before the ingestion of the first pill at the initiation of oral contraceptive use and to the levels determined 24 hours after ingestion of the pill for day 20 at the 3-, 6-, and 9-month intervals. The initial slope was calculated by means of this 0-hour level value and the level determined 1 hour after ingestion of the pill for day 21 (i.e., 1-hour level minus 0-hour level over the 1-hour time interval). Subjects did not always have sampling precisely at these intervals; therefore actual times were used in the calculation.

We used a variety of statistical techniques to analyze the resultant data, including t and χ^2 tests, split-plot repeated measures analysis of variance, multiple linear regression analysis, multiple logistic regression analysis, and correlational analysis. In addition, we developed a procedure called trichotomous regression for use in analyzing the change in percent outliers from baseline to 3, 6, or 9 months, which takes into account the paired nature of the data and permits the evaluation of independent variables.[14]

Results

From Oct. 1, 1985, through Oct. 15, 1987, 316 women were enrolled in the study. Forty-seven withdrew or were excluded after the initial interview and 18 were excluded or withdrew after the initial physical examination. Because of inconvenience, minor side effects, or failure to keep appointments, 35 women withdrew between the first pill cycle and the 3-month visit, 11 women withdrew between the 3- and 6-month visits, and 13 women withdrew between the 6- and 9-month visits. A total of 192 women completed the full 9-month study cycle and are included in the subsequent analyses.

After randomization into the three oral contraceptive groups, there were no significant differences in respect to age, weight, body mass, prior oral contraceptive use, gravidity or parity, race, menstrual pattern, marital status, cigarette smoking, exercise, diet, alcohol intake, and past severe illness. The 59 eligible women not completing the full study cycle did not vary significantly from the women completing the study, except they weighed an average 2.4 kg less ($p = 0.04$) than the study group. These women were evenly distributed among the three study groups. The mean age in each group was about 25 years, and the mean weight was about 61 kg. A more detailed analysis of the study group characteristics has been presented elsewhere.[14, 15]

The resultant randomization and subsequent with-

Table I. Mean percentages for antithrombin III at baseline and at 3, 6, and 9 months for all oral contraceptive groups combined*

	Baseline	*3 mo*	*6 mo*	*9 mo*
Immunologic method	121.58 ± 1.89	101.09 ± 1.78	98.75 ± 1.89	97.66 ± 2.00
Activity method	129.42 ± 1.50	105.68 ± 1.86	98.27 ± 2.19	92.14 ± 2.29

*Values are mean ± SE. $p < 0.0001$ by repeated-measures analysis of variance.

Table II. Mean percentage decrease in antithrombin III (activity method) by oral contraceptive type and ABO blood group*

Blood type	*No.*	*0-3 mo*	*0-6 mo*	*0-9 mo*	*Combined mean*
50 μg Ethinyl estradiol, 1.0 mg norethindrone					
O	31	25.7 ± 5.5	30.7 ± 5.7	38.4 ± 6.9	31.6
Non-O	36	21.8 ± 5.0	25.2 ± 6.2	29.0 ± 5.6	25.3
35 μg Ethinyl estradiol, 1.0 mg norethindrone					
O	25	23.6 ± 5.8	27.7 ± 6.0	31.1 ± 7.2	27.5
Non-O	36	16.4 ± 5.0	24.2 ± 5.0	48.1 ± 4.7	29.6
35 μg Ethinyl estradiol, 0.5 mg norethindrone					
O	23	24.8 ± 8.2	38.1 ± 7.1	25.2 ± 7.0	29.4
Non-O	40	30.6 ± 4.0	41.5 ± 5.1	44.5 ± 7.0	38.9

*Values are mean ± SE. $p = 0.012$ by repeated-measures analysis of variance for interaction between blood group and oral contraceptive type.

drawals resulted in study groups composed as follows: 50 μg ethinyl estradiol plus 1.0 mg norethindrone, 67 women; 35 μg ethinyl estradiol plus 1.0 mg norethindrone, 61 women; and 35 μg ethinyl estradiol plus 0.5 mg norethindrone, 64 women.

Table I describes the changes in antithrombin III values for both methods for the combined oral contraceptive groups over the 9-month study period. There were no statistically significant differences according to type of oral contraceptive preparation. Mean baseline antithrombin III values for the immunologic and activity determinations, respectively, by ABO blood group type were: type O, 120.4% and 126.5%; type A, 120.3% and 130.8%; type B, 122.2% and 127.5%; and type AB, 113.6% and 131.9%. Table II presents changes in antithrombin III by the activity method according to oral contraceptive type and whether ABO blood type was O or non-O. These effects persisted even when the data were adjusted for other possible covariables, such as baseline antithrombin III. A similar analysis using the immunologic method showed declines in each time interval but no significant differences among oral contraceptive preparations or according to ABO blood type.

A number of trends were also shown when the antithrombin III data and contraceptive steroid values were related to outlier status. For the immunologic method, change in 0-hour ethinyl estradiol over the 9-month study period was positively associated with outlier status ($p = 0.01$); that is, higher estrogen levels before a subject ingested her next contraceptive pill were associated with an increased likelihood that she would achieve outlier status for antithrombin III. Change in the initial slope of ethinyl estradiol from baseline to 3 months was negatively associated with outlier status ($p = 0.02$); that is, the greater the change in slope between the two time intervals, the less likely a subject achieved outlier status for antithrombin III. In addition, the baseline initial slope of norethindrone was also positively associated with change to outlier status ($p = 0.03$). Finally, the higher the baseline immunologic antithrombin III measurement, the more likely a subject was to achieve outlier status at 9 months ($p = 0.0002$). None of these findings were significantly affected by type of oral contraceptive preparation.

For the activity method, the baseline initial slope of ethinyl estradiol and change in initial slope of ethinyl estradiol over time were negatively associated with change to antithrombin III outlier status ($p = 0.04$ and $p = 0.01$, respectively); that is, higher initial slopes or greater changes in slope over time for ethinyl estradiol reduced the likelihood that subjects would eventually achieve outlier status. Finally, the higher the activity antithrombin III level at baseline, the more likely a subject was to achieve outlier status at any subsequent time interval ($p < 0.001$). As with the immunologic method, there were no statistically significant differences shown according to oral contraceptive type in these analyses.

Comment

Thromboembolism is the most frequent serious sequela of the cardiovascular system associated with oral contraceptive use.[11] Although recent data suggest that this sequela may be decreasing in frequency as a result of lower-dose preparations, the relative risk of thromboembolism among oral contraceptive users is still about threefold.[23] Numerous studies have been undertaken to try to explain this association on the basis of underlying changes in the coagulation system; however, results have varied and to date none have been predictive.[10]

Since a congenital deficiency of antithrombin III has been shown to be associated with a high frequency of thrombosis, much attention has been focused on evaluating it in oral contraceptive users.[8, 11] However, several points need to be kept in mind. First, plasma levels of antithrombin III range between 40% and 55% of normal in families in whom the congenital deficiency has been described. However, not all family members exhibit thrombosis with these levels; in fact, antithrombin III levels in such families are not very predictive of clotting risk.[8] Among individuals who do not have the congenital deficiency but who do have thromboembolism, one author[24] estimated that approximately 70% have decreased levels of antithrombin III before initiation of anticoagulant therapy. However, it should be noted that these levels primarily reflect consumption that is due to thrombus development rather than preexisting deficiency.

The technique by which antithrombin III is measured appears important. Serum measurements may not reflect the true status, since the test systems measure the inactivation of thrombin, not the quantity of antithrombin III.[10] For example, elevations of prothrombin (often seen in oral contraceptive users) can result in declines in antithrombin III levels when serum measurements are used. Most authors[10, 20] accordingly recommend measurement in plasma to avoid this problem. Finally, it has been suggested that both an immunologic method and an assay for activity be used to better evaluate the effect of oral contraceptives on antithrombin III.[10, 11] Immunologic assays estimate overall quantity of antithrombin III but not necessarily activity. For example, some assays have been shown to give positive results in the presence of inactive protein. Activity assays that are based on the inhibition of factor Xa are independent of the rate of factor Xa inhibition, are not affected by large amounts of heparin in the test plasma, and are independent of other naturally occurring plasma protease inhibitors.[10, 20]

ABO blood group has been shown to be associated with risk of thromboembolism.[11, 17] Specifically, individuals with type O blood who are using oral contraceptives appeared to be relatively protected, compared with individuals with other blood types in one collaborative study.[17] For example, the relative risk of thromboembolism for oral contraceptive users with non-O blood type as opposed to blood type O was 3.2. In addition, one study also showed that women with blood types other than O (particularly type A) had lower antithrombin III levels when measured by an immunologic method.[16] Therefore a potential explanation for the interrelationship between ABO blood type and oral contraceptive use relative to thromboembolism could be reduction in levels of antithrombin III.

This study examined these two potential risk factors for thromboembolism (i.e., levels of antithrombin III, ABO blood type) among oral contraceptive users according to dosage and metabolism of the contraceptive steroids. Similar to other published results, mean levels of antithrombin III decreased over the study interval from baseline levels by 19.7% and 28.8% for the immunologic and activity measurements, respectively.[1-7] In addition, the data presented in Table I also suggest that these values could continue to decline beyond the time interval of the current study. A similar trend has been observed in other studies as well.[1, 6] The significance of this finding is at best unclear. If antithrombin III values were to continue to fall and if low values in oral contraceptive users were related to thromboembolic risk, one might expect to see duration of use as a risk factor for thromboembolism. However, available epidemiologic data does not suggest that there is a duration effect for thromboembolism among oral contraceptive users.[11]

When baseline levels of antithrombin III were examined in relation to blood group type, there were no significant differences shown regardless of methodology. Therefore were were unable to confirm previous findings, although perhaps the sample sizes in our study were too small to fully investigate this particular point.

The data presented in Table II do suggest an interaction between blood group type and type of oral contraceptive. For example, it appears that women with type O blood who use the 50 μg estrogen preparation showed greater declines in antithrombin III levels than those with non-O blood or those receiving a 35 μg estrogen oral contraceptive containing the same dose of progestin, i.e., 1.0 mg norethindrone. In addition, women receiving the lowest dose of norethindrone, i.e., 0.5 mg, who were blood group type non-O also showed larger declines in antithrombin III. These differences were only shown with the activity method. Although prior literature has indicated that the degree of change in antithrombin III better correlates with estrogen dose, at least one study involving the progestin lynestrenol has shown decreased levels of antithrombin III.[13] However, the authors of that article suggested that the estrogenic biologic activity of that compound was responsible for its actions on antithrombin III. Although there is no clear explanation regarding the differences seen according to oral contraceptive type and blood group type in this study, there are some possibilities. These differences may mean that the estrogen or progestin component interacts differently with individuals with O or non-O blood group type through some mechanism that has yet to be defined. Alternatively, particularly when one examines the oral contraceptive containing 35 μg ethinyl estradiol and 0.5 mg norethindrone, when the doses in oral contraceptives achieve low levels, their effects on antithrombin III are minimal in comparison with other factors. Therefore for this particular oral contraceptive only the differences or activity related to blood type are operant. Obviously, confirmation with additional studies would be of importance.

The metabolic changes of the contraceptive steroids and change to outlier status have some areas of consistency. The change in initial slope of ethinyl estradiol was negatively associated with change to outlier status for both types of antithrombin III determinations. This finding suggests that rapid uptake of the estrogen in some way reduces the chance that an individual will show more marked declines in antithrombin III. For the immunologic method, a high 0-hour ethinyl estradiol level was correlated with change to outlier status, a finding somewhat supporting the contention that estrogen dose correlates with the degree of antithrombin III fall among oral contraceptive users. The correlation between high initial antithrombin III levels and subsequent change to outlier status is difficult to explain. It may mean that such individuals are more sensitive to the effects of contraceptive steroids or that antithrombin III is catabolized or more rapidly cleared from the blood.

The findings in this study suggest that interactions among potential risk factors for thromboembolism are complex. However, further detailed study of these risk factors could yield useful information that might provide a better understanding of the risk of these disorders among users of oral contraceptives. Although it is unlikely that screening all oral contraceptive users for thromboembolism with a coagulation factor study such as antithrombin III will ever prove practical or cost-effective because of the overall low risk of this problem, screening certain subgroups might be important. For example, in one study of former oral contraceptive users who had experienced a cerebrovascular accident, the presence of reduced antithrombin III levels and mitral valve prolapse was striking when these women were compared with a control group.[25] Thus an area of future investigation could be the determination of antithrombin III levels in women with mitral valve prolapse before and during oral contraceptive use to detect changes that might suggest increased risk of thromboembolic cerebrovascular morbidity.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Dennis J. Lutz, Minot, North Dakota. After the general availability of oral contraceptives in the early 1960s, evidence rapidly accumulated suggesting that the "pill" had effects on health other than the prevention of unwanted pregnancy. The epidemiologic evaluation of oral contraceptives began with the issue of thromboembolic disease and has been greatly expanded during the past 30 years. In particular, antithrombin III was noted very early to diminish in women taking oral contraceptives. Because individuals with congenital antithrombin III deficiency have a strong tendency to thromboembolic disease and because low antithrombin III levels are frequently observed in patients with thromboembolic disease, the opportunity seemed obvious for a presumably neat and tidy area of scientific investigation.

In 1971 von Kaulla et al.[1] showed that 80 μg estrogen–containing oral contraceptives induced a depression of antithrombin III activity plus an acceleration of thrombin generation almost immediately within the first cycle of therapy. Since these changes were very similar to those observed in patients with chronic thromboembolic disease, it was suggested that of all the clotting changes induced by oral contraceptives the loss of antithrombin III activity may be the one of the most clinical significance.

By 1980 it was apparent that oral contraceptives containing 75 to 150 μg of estrogen appeared to decrease antithrombin III activity to a greater extent than did the 50 μg dosage, while most progestogens alone did not appear to alter antithrombin III activity. Quantitative levels of antithrombin III were also noted to be lower in women with non-O blood types. A decade ago Stadel[2] therefore questioned whether oral contraceptive use is accompanied by a critical decrease in antithrombin III activity more frequently in women of non-O blood types. Dr. Burkman and his colleagues are now trying to address this question as another decade begins.

This article is a continuation and elaboration of Dr. Burkman's efforts to define the relationship of oral contraceptives to several clinical and laboratory parameters over time. He has successfully demonstrated and confirmed that both immunologic and activity levels of antithrombin III decrease within 3 months of initiation of oral contraceptive therapy. Although immunologic levels declined and stabilized at the end of the 9-month study, the more clinically important activity levels were still decreasing at 9 months. Perhaps an extension of this study to include 12-, 15-, and 18-month data would have been more appropriate? Since there were no statistically significant differences according to the type of oral contraceptive preparation prescribed, can any conclusions be drawn regarding dosage of estrogen or progestogen? Is a similar study being considered for triphasic preparations that were not readily available when this study began in 1985 but which have today become the oral contraceptive of choice for most patients?

Burkman et al. were unable to confirm any difference in baseline antithrombin III levels on the basis of blood group type. Perhaps the study group was too small, as they suggested, or perhaps earlier reports are incorrect. The mean percentage decrease in antithrombin III activity by oral contraceptive type and ABO blood group is intriguing but still difficult to analyze, again because of the small sample size in each subgroup.

Dr. Burkman and his colleagues are to be congratulated on using the randomized trial approach and for expending considerable attention to techniques that accurately measure both the immunologic and activity levels of antithrombin III. This has not always been done in the past and probably accounts for much of the conflicting data in the literature. Above all, the unbelievable complexity of the human coagulation mechanism is highlighted by this article, which raises more questions than it answers.

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Dr. Ramona I. Slupik, Chicago, Illinois. This article generates more questions than answers. The variety of potential risk factors for thromboembolic disease explored here makes for some difficulty in reaching a conclusion that is clinically applicable, as the authors have admitted.

The most critical questions here today are: (1) Is oral contraceptive use accompanied by a biologically significant decrease in antithrombin III to <50%? (2) Is this decrease more likely to occur in women with non-O type or with certain oral contraceptive formulations? There still seems to be no test or combination of tests that can reliably predict in which patient a thromboembolic event may develop in association with oral contraceptives.

A reduction in antithrombin III activity of ≥50% substantially increases the risk of overt venous thromboembolic disease, and a reduction of ≥20% can be predictive of subclinical venous thromboembolic conditions extensive enough to be detectable by iodine 125–fibrinogen uptake.[1]

Obviously sole use of decreased activity levels of antithrombin III in oral contraceptive users cannot adequately predict the chance that overt thromboembolic disease will develop. Even in families with congenital antithrombin III deficiency where levels fall below 50%, not all individuals demonstrate thromboembolic disease, although up to 85% of these individuals do have a thromboembolic event between the second and fourth decades, when oral contraceptive use would be most likely.[2] Furthermore, antithrombin III decrements of this extent were not documented in this study. α_2-Macroglobulin and α_1-antitrypsin levels, with their contradictory ability to inhibit both thrombin and plasmin, are increased in oral contraceptive users and may play a modulatory role here[3,4]; varying degrees of decreased plasminogen activator release also may be involved.[5] The degree to which the oral contraceptives influence levels of the anticoagulants plasma protein C and protein S is yet to be elucidated.[6]

The role of ABO blood type creates some fascinating confusion here. In women not using oral contraceptives the risk of idiopathic deep venous thromboembolic disease is twice as great in patients with type non-O versus those with type O,[7] which may be due in part to lower levels of factor IX (von Willebrand's factor) in type O patients.[8] In oral contraceptive users the increased risk of deep venous thrombosis for women with type non-O is threefold,[7] suggesting a genetic susceptibility to idiopathic deep venous thrombosis that is multiplied by oral contraceptives.

Another potential explanation for these differences in risk is the relatively lower antithrombin III levels seen in type non-O (specifically type A), as compared with type O.[9] These variations in baseline antithrombin III levels between blood-type groups were not borne out in the present study, and the authors admit that small sample size may be the reason. The reason that all baseline antithrombin III levels in this paper were >100% is also unclear, because the authors refer to a normal activity level as 55% to 100%. Likewise, the "normal" immunologic antithrombin III value of 71% to 200% is not explained by the references cited[10, 11]; methods used by our hematology research laboratory at Northwestern University ascribe a normal value as 71% to 110% (unpublished data). Do these elevated baseline antithrombin III levels seen here reflect a nonrandom population sampling?

Another question relates to the changes in antithrombin III levels in type O and non-O subjects, relative to the three different oral contraceptive preparations studied. If variations in deep venous thrombosis incidence with ABO blood group presume a genetic susceptibility, who the diverse response to the oral contraceptive formulations? Whereas we would have expected to see both groups of women do worse on the higher estrogen pill, we instead see the more significant drop in antithrombin III in the patients with blood type O, previously regarded as somehow protective. Furthermore, the non-O patient, traditionally considered to be at higher risk, especially at higher estrogen levels, has a greater drop in antithrombin III on the lower estrogen formulation and a still greater drop when the progestin also is decreased. Does the progestin exhibit some type of protective effect? Is it dose-related? Does the mechanism pertain to relative estrogenic procoagulant biologic activity[12]? Is the progestin effect significant only in non-O patients? (Even so, the type O individuals also fared better on the higher progestin pill.) Perhaps presenting Table II as a scatter diagram, with absolute antithrombin III values and serial decreases instead of mean percentage decreases, would provide more relevant information regarding the number of individuals in whom a clinically significant reduction in antithrombin III was reached.

I have some questions. Should a patient's blood type influence which oral contraceptive we prescribe for her? Determination of blood type is not unreasonable and is certainly more economically feasible than testing baseline antithrombin III, which would detect only individuals at risk because of hereditary antithrombin III deficiency, or 1 of 2000 to 1 of 20,000 patients.[2] However, because we know antithrombin III levels must decrease by ≥50% to be clinically significant, can we justify any testing on the basis of the minor decreases reported in this article? Or should we test only certain subgroups known to be at higher risk, such as those with a family history of thromboembolic disease?

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Dr. James R. Scott, Salt Lake City, Utah. Congenital or acquired clotting abnormalities, whether antithrombin III, protein C, or lupus anticoagulant, are very rare. Are the decreases in antithrombin III as you describe actually clinically important? In other words, has it been shown that patients with decreased levels in this range have an increased incidence of thrombotic episodes? I didn't hear anything about the clinical characteristics of these patients. Did any have a history of thrombosis?

Dr. Burkman (Closing). I thank Dr. Lutz and Dr. Slupik for their comments, as well as those who commented from the floor. I think both of the formal discussants have pointed out the complexities of studying this issue. What we are trying to do is relate a measurement of some type of clotting or fibrinolytic factor to the occurrence of a clinical event.

In the case of oral contraceptive users, the type of event, thromboembolism, occurs roughly between 50 and 100 times per 100,000 users per year. Therefore, relative to the question raised from the floor, the likelihood of someone in a study group actually experiencing the event one is trying to predict is low; that is, as one might predict, no one in this study had thromboembolism.

As to the predictability of low levels of antithrombin III, Dr. Slupik pointed out that even in families with congenital deficits with values of 40% to 50% of normal, a sizable proportion of individuals within the families may, in fact, have low levels and still not experience thromboembolism. Therefore, even with congenital deficiency, antithrombin III levels are not highly predictive.

The problem I think we are facing is trying to identify some proxy for thromboembolism. It is probably a problem of trying to use values determined in the clotting or fibrinolytic system, when there is a third factor that is difficult to study, the surface where the clot occurs. For example, that is why the data relating some of the changes in antithrombin III in women with mitral valve prolapse seem interesting to me as a means of studying the role of this third factor.

In regard to some of the other questions raised, we have not repeated this study with triphasic preparations. Although it is not clear why we did not show baseline antithrombin III differences among blood groups, it may very well be related to the methods used to perform the measurements or the small sample sizes in this study. The question was raised as to why some of the baseline values were above normal. This could mean that for some reason the women recruited into this study were in some way different from the general population, or it may be that the pools of women used for deriving the normal values were somewhat different. In any event we thought that the changes over time were of greater importance then the absolute values. Should blood group type influence what oral contraceptive you prescribe for a patient? I do not think so. This study was intended to show a method that might be useful for developing a hypothesis or means of studying small subgroups of women at risk. However, for the larger population of oral contraceptive users, I don't think any of these techniques should be used at the present time.

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Should cervical cytologic testing be augmented by cervicography or human papillomavirus deoxyribonucleic acid detection?

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Criticism of the Papanicolaou smear in the lay press and recent federal legislation regulating cytology laboratories indicate a need to reappraise cervical cancer screening programs. This study directly compares three potential screening tests, used alone or in combination. A total of 1012 women aged 18 to 35 years were screened by cytologic testing, cervicography, and hybridization for human papillomavirus deoxyribonucleic acid, with discrepancies being refereed by the last two authors. After findings from the entire lower genital tract were combined, 116 women (11.5%) showed definite clinical abnormalities (either exophytic vulvovaginal condylomas or cervical squamous intraepithelial lesions). Another 72 (7.2%) had positive Southern blot hybridizations without accompanying viral expression, yielding a cumulative frequency for established disease or latent infection of 18.6%. When associated vulvovaginal condylomas are disregarded, final groupings with regard to cervical pathologic classification were: 23 high-grade and 71 low-grade squamous intraepithelial lesions, 164 cases of equivocal atypia (34 of which had detectable human papillomavirus deoxyribonucleic acid), and 754 cases with negative results (38 of which had detectable human papillomavirus deoxyribonucleic acid). Cervical screening tests were compared principally by plotting increasingly liberal recall criteria onto receiver operating characteristic curves (i.e., graphs of true-positive results on the Y axis versus false-positive results on the X axis). Used individually, each screening test was valid, but none was substantially better than the others. No matter how liberal the recall criteria, no single test was able to detect all of the 23 definite precursors in this sample. Applying conventional recall criteria (i.e., high- or low-grade lesion suspected), cytologic testing alone detected 12 high-grade squamous intraepithelial lesions (52.2%), at a cost of having to perform colposcopy in 8.7% of the sample. Combining all three tests and setting the end point as just a high-positive result by at least one test, 19 high-grade squamous intraepithelial lesions (83%) were detected, with a recall of 7%. Optimal test performance (96% sensitivity, 4% recall) would have been attained by recalling all patients with high-grade cytologic results or positive cervicography results, plus any patients with low-grade morphologic atypia in which hybridization detected an oncogenic human papillomavirus type. Our conclusions are as follows: (1) Cytologic detection rates are markedly improved by a second or third test; (2) increased screening costs could be offset by not recalling patients with minor lesions with no apparent potential for progression. (Am J Obstet Gynecol 1991;164:1461-71.)

Key words: Cervical neoplasia, cytology, cervicography, HPV testing, cost-effectiveness

Screening tests are relatively simple procedures designed to separate well persons from those with a high probability of having the disease under study, so as to permit early diagnosis and treatment. Test validity is measured in terms of two reciprocal ratios: sensitivity (positivity in disease) and specificity (negativity in health). Since the distribution of healthy and diseased subjects generally overlaps, a single screening test can never be perfect.[1] Rather, the cutoff point must be adjusted to compromise between maximizing sensitivity, at the cost of recalling some healthy subjects (false-positive results), or maximizing specificity at the cost of overlooking some subjects with disease (false-negative results). Highest specificity is important (1) where the disease in question is serious but not readily treatable, (2) where knowledge as to absence of disease has important personal and public health values, and (3) where false-positive results have serious sequelae (e.g.,

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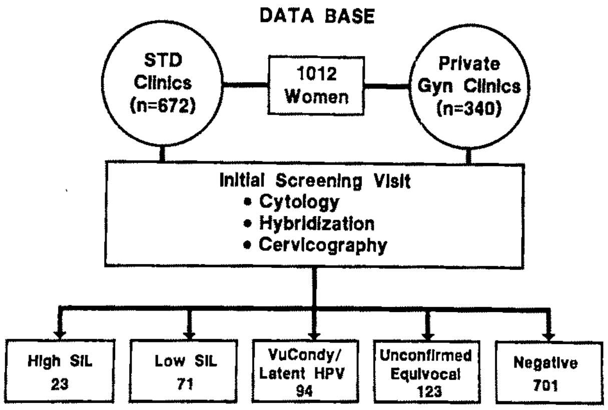

Fig. 1. Diagram of research architecture and basic group assignments within this sample of 1012 women.

screening for human immunodeficiency virus infection). Conversely, sensitivity is accentuated (1) when early stages of the disease are readily treatable, (2) when missed diagnosis can squander the prospect of cure, and (3) when false-positive results do not create serious psychologic or economic hardship (e.g., colonic screening for occult blood). Since criteria for interpreting Papanicolaou smears were formulated in the precolposcopy era, at a time when a positive result led to cold knife conization and possible hysterectomy, cytologic cutoff points have favored specificity over sensitivity.[2] Adequacy of disease detection has traditionally relied on the likelihood of detecting false-negative cases at follow-up screening examinations, before progression to invasion cancer could occur.[3] However, widespread lay acceptance that cervical cancer is an avoidable disease has made apparent malfunction of the screening process a highly litigious area of gynecology.

In addition to being valid and acceptable, screening tests must also be justifiable on economic grounds. Cost/benefit ratio is generally measured in terms of the positive predictive value (the proportion of positive results that are true positive, after the test is applied to a population of both healthy and diseased subjects). Whereas sensitivity and specificity are constant for a given test, predictive values change dramatically according to disease prevalence within the screened population.[1] Since false-positive results always occur at a relatively constant rate, positive predictive value will either soar or plummet as the frequency of true-positive results rises or falls. Moreover, positive predictive value is more closely related to specificity (the ability to avoid false-positive results) than to sensitivity (avoidance of false-negative results). Therefore cost-effectiveness of cervical screening programs has been tied to the traditionally high specificity of the Papanicolaou smear. Introduction of the Bethesda terminology,[4] with its tendency to increase recall of patients with minor grade atypia, carries a risk of eroding the traditionally strong positive predictive value of an abnormal Papanicolaou smear.

After four decades of experience with exfoliative cytology, it seems doubtful that new advances can be secured by adaptations of the Papanicolaou smear alone. Rather, the best prospect of further reducing cervical cancer among screened women probably will depend on the incorporation of adjunctive measures such as cervicography or testing for human papillomavirus (HPV) infection. In that "oncogenic" HPV types are now incriminated as a cause of squamous intraepithelial lesions that may progress to cervical cancer,[5] viral testing would appear to hold considerable promise. Alternatively, even if the apparently causal association between "oncogenic" HPV infection and cervical neoplasia should subsequently be disproved, the strength and specificity of this association would still point to HPV detection as having valuable potential as a "surrogate" test.[6] However, to date, the clinical value of testing for the simple presence of HPV deoxyribonucleic acid (DNA) has been clouded by the fact that in 10% to 25% of apparently healthy women, these agents are exfoliated in morphologically normal cells.[7,8]

Whether such latent infections demarcate a large "at risk" pool or simply represent colonization of individuals who are not presently susceptible to disease expression remains an open issue. Even if it is accepted that testing for HPV DNA will not function as a useful primary detection tool, there is still a good possibility that HPV testing of minor grade lesions might perform well as an intermediate triage strategy.[9] Likewise, although formal colposcopic examination is too laborious for use as a rapid screening test, a modification of this technique by means of the cerviscope camera solves the logistic difficulties confronting screening colposcopy.[10] Hence this study was designed to compare available screening tests in a high-risk population, to examine type-specific viral associations, to approximate the prevalence of HPV exposure in both high- and low-risk groups, and to explore new options for improved cervical screening. Data analysis has centered primarily on using these data as a theoretic screening model, within which the practical consequences of different diagnostic decisions can be weighed.

Material and methods

Study design. This prospective survey examined the prevalence of lower genital tract HPV infection in a structured population of 1012 urban and suburban women aged 18 to 35 years, recruitment being undertaken in both a sexually transmitted disease (STD) clinic and private gynecologic offices. Patients were then screened for HPV infection and cervical intraepithelial neoplasia, by means of cytologic testing, cervicography, and DNA hybridization of exfoliated cells. The 298 women having an abnormal result by any one test were recalled for colposcopy and targeted biopsy. However, 68 of the subjects attending the clinic were either untraceable or unwilling to return for colposcopy. Since omitting these 68 women would have changed the balance between positive and negative screening test results, this group was retained under an assigned diagnosis.

Socioeconomic, clinicopathologic, and virologic results were entered into a computerized data base, cross checked for accuracy, and analyzed with the BMDP-88 statistical packages within an IBM386-2 microcomputer. Standard statistical differences were analyzed by the χ^2 test. Comparison of the different cervical screening tests was done principally by receiver operating characteristic curves, generated by plotting true-positive results on the Y axis and false-positive results on the X axis. Receiver operating characteristic curves have two major advantages. First, differing validities and efficiencies of multiple tests are compared graphically. "Useful" tests produce a linear plot with a slope of 45 degrees ("coin flip"), "perfect" tests produce a horizontal linear plot originating from 1.0 on the Y axis, and real-life tests produce curved plots lying within the upper left triangle (formed between the plots for a "useless" and a "perfect" test). Quality of test performance is judged by slope (vertical indicating good cost-effectiveness and horizontal indicating inefficiency) and proximity to the Y axis. Second, receiver operating characteristic curves allow ready visual comparison of the relationship between sensitivity and predictive value, as the cutoff points for any given test (or combination of tests) are variously restricted or liberalized.

Study population. The study population was drawn from two groups: a "high-risk" and a "low-risk" subset. The "high-risk" sample consisted of 672 volunteers attending Herman Keifer Clinic for Sexually Transmitted Diseases. The "low-risk" portion comprised 340 volunteers, recruited from four different gynecologic clinics to be more representative of affluent and less affluent women, both white and black (Fig. 1). Eligibility was restricted to patients aged 18 to 35 years, who had not presented for treatment of either cervical dysplasia or vulvar warts. Pregnant women, those who had undergone hysterectomy, and anyone taking immunosuppressant drugs were excluded. After informed consent was obtained, patients were interviewed for ascertainment of basic demographic and clinical information.

Median age was 24 years, and 621 (92%) were black. Distinct differences existed between sites of recruitment. Patients attending the STD clinic had the earliest median age of first intercourse, the highest lifetime number of sex partners, the highest median number of different sex partners per year of sexual activity, the highest proportion of Afro-Americans, and the highest proportion who reported a previous sexually transmitted infection (Table I). Hence, with regard to the intent to recruit subsets of women with differing levels of exposure to sexually transmitted infections, our sampling methods apparently achieved the study objectives of generating "high-risk" versus "low-risk" subsets. Demographics and sexual behavior reflected the heavy recruitment from the STD clinic. Of 999 respondents to questions on previous sexual behavior, 987 (98.8%) commenced coitus by age 20, and 819 (82%) reported three or more lifetime sexual partners. When these two variables were combined for evaluation of the American Cancer Society's definition of the "risk of abnormal cervical cytology,"[11] only 17 individuals (1.7%) fell into the "low-risk" category.

Table I. Demographic, sexual, and socioeconomic differences between the "high-risk" and "low-risk" subsets within this sample of 1012 women

Variable of interest	*STD clinic*	*Routine gynecologic clinics*
Proportion of black patients	621 (92%)	115 (34%)
Proportion of college graduates	225 (33%)	149 (48%)
Median age at first intercourse (yr)	16	17
Median lifetime No. of sex partners	6	3
Median No. of sex partners per year	2	1
Previous STD history	225 (33%)	59 (17%)
TOTAL	672	340

Screening examination. After careful inspection of the external genitalia, the cervix was exposed with an unlubricated speculum, and appropriate microbiologic studies were obtained (wet mount and gonococcal cultures). Cytologic screening was done with a two-slide technique—collection of an endocervical sample with a Zelsmyr Cytobrush and an exocervical sample with a dagger spatula. Results were reported as high-grade positive (cervical intraepithelial neoplasia grade 2 or 3 or higher), low-grade positive (definitely abnormal, suggestive of well-developed koilocytotic atypia or cervical intraepithelial neoplasia grade 1), equivocal (minor dyskaryosis of uncertain significance), or negative (no features of HPV infection). All positive and equivocal smears, together with a 10% sample of normal ones, were examined independently by two cytopathologists (M.H. and A.B.J.). Any discrepancy between

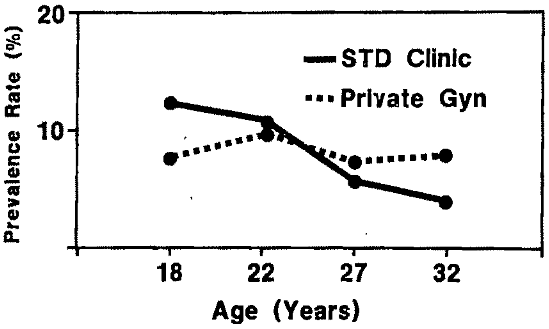

Fig. 2. Comparison of age-specific detection rates (for condyloma or cervical intraepithelial neoplasia), broken down by site of enrollment (*solid line*, STD clinic; *dashed line*, gynecology clinics).

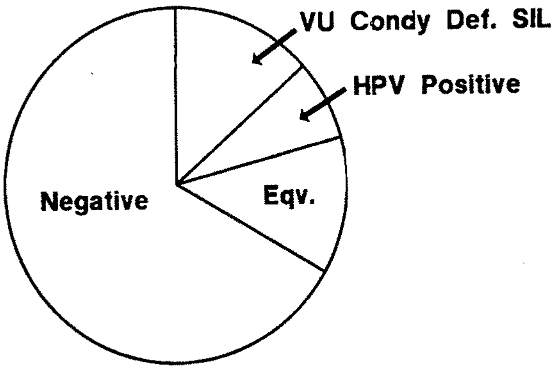

Fig. 3. Pie chart showing cumulative frequency of clinically expressed cervical or vulvar HPV-associated disease (11.5%), latent cervicovaginal HPV infection (18.6%), and the finding of an equivocal cervical screening test (30.7%) within this sample of 1012 women.

the paired cytopathologic opinions were adjudicated by a third cytopathologist (C.R.L.).

After the Papanicolaou smear was completed, the cervix was swabbed twice with 4% acetic acid and then photographed. Initially, photographs were taken with a standard camera fitted with bellows and a macrolens. However, resolution was poor and focus unreliable. The presence or absence of a lesion could be recognized, but grading was difficult. Hence after 3 months photography was done with a commercially available cerviscope, this being the best means of ensuring reproduction of the entire cervix, with minimal blur, excellent depth of field, and optimal color contrast between different tones of red. Images were interpreted by subsequent projection onto a 10-foot screen, with the observer standing at a distance of 3 feet. By this means, apparent magnification was comparable to the ×16 enlargement used during colposcopic examination. Results were reported in four categories: positive for a definite lesion showing high-grade features, positive for a definite lesion showing low-grade features, acetowhite change of uncertain significance, or negative.

The third screening method involved placing the residual cellular material from the Cytobrush and spatula within a special transport solution, for subsequent DNA extraction and Southern blot hybridization with radiolabeled probes for HPV types 6, 11, 16, 18, 31, 33, 35, 42, 43, 44, 45, 51, 52, and 56. Results were grouped as positive for an oncogenic type (HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56), positive for a low-risk type (HPV types 6, 11, 42, 43, and 44), positive for an unknown type, or negative.

Diagnostic evaluation of positive screening test results. The raw data were unexpectedly difficult to interpret, because of the following complexities: (1) Although the 343 definitely abnormal cervical test results (88 cytologic results, 135 cervigrams, and 120 hybridizations) were concentrated among 298 patients, the same clarity of demarcation did not hold for lesser degrees of atypia. Almost one third of the sample had an equivocal result by one or another of the screening tests. (2) Of the 298 patients to be recalled for colposcopy and directed biopsy, 68 were either untraceable or unwilling to return for diagnostic assessment. Since excluding this subset would have altered the balance between positive and negative screening patterns, these patients were retained and assigned to one of the diagnostic groups on the basis of the available information (Fig. 1). Such diagnoses were always conservative, with doubtful cases being downgraded to a lower category. Nonetheless, 17 women with definite abnormalities on a least two of the four examinations were classified as showing positive results: Three women were designated as having high-grade lesions and 14 as having low-grade lesions. Because the results of this study are mainly concerned with measuring how often the 23 high-grade lesions were either detected or missed by the various tests, the three women assigned to the high-grade category are the only ones with assumptions of any significance. The inclusion of 14 cases as low-grade lesions was of importance only in estimating the number of patients who, under conventional rules of diagnostic triage, would be recalled for biopsy and possible empiric therapy. (3) Even among those who had adequate assessment, 66 biopsy specimens showed histologic appearances that fell into the realm of nonspecific epithelial changes, rather than unequivocal koilocytotic atypia (Fig. 1). To avoid either overemphasizing or underemphasizing the importance of these equivocal histologic patterns, they were retained as a separate category that could be collapsed or maintained, according to the question of interest.

Results

Prevalence of HPV infection. After cervical and vulvar findings were combined, final status with regard to

Table II. Combined cervical, vaginal, and vulvar abnormalities among 1012 women

HPV status	*Category total*		*Cumulative count*	
	No.	*%*	*No.*	*%*
Vulvar condylomas* (possible cervical lesion)	31	3.1	31	3.1
Definite cervical lesion* (possible vulvar condylomas)	94	9.3	116	11.5
Positive HPV DNA but no definite lesion	72	7.1	188	18.6
Equivocal cytology/cervicography but negative HPV DNA hybridization	123	12.2	311	30.7
Negative by all tests	701	69.3	701	69.3
TOTAL	Categories not mutually exclusive*		1012	100

*Nine women with vulvar condylomas also had definite, low-grade squamous intraepithelial lesions of the cervix. Hence cumulative prevalence of expressed disease was 31 + 94 − 9 = 116.

HPV infection was as follows: 31 cases of exophytic vaginal or vulvar condyloma acuminatum (9 of these patients also had low-grade cervical lesions), 23 high-grade (cervical intraepithelial neoplasia grade 2 or higher) and 71 low-grade cervical squamous intraepithelial lesions (9 of these patients had vulvovaginal condylomas), 72 positive hybridizations without definite clinical expression, 123 equivocal cervical screening test results that were not confirmed by Southern blot hybridization, and 701 with negative results by all four examinations. If associated vulvovaginal condylomas are disregarded, morphologic findings with respect to cervical disease were: 23 high-grade squamous intraepithelial lesions, 71 low-grade squamous intraepithelial lesions, 164 equivocal cytologic or cervicographic screening test results (34 of these patients had detectable HPV DNA), and 754 morphologically normal cervices (38 of these patients had detectable HPV DNA).

Patients recruited at the STD clinic differed significantly with regard to age at first intercourse, lifetime number of sex partners, number of sexual partners per year, educational level, and income (Table I). Nonetheless, the only disease manifestation seen more frequently in the high-risk group was that exophytic vulvar condylomas were three times more common at the STD clinic (3.9% vs 1.5%). The age-specific detection rates for cervical condyloma or cervical intraepithelial neoplasia were the same for both subsets (Fig. 2). Moreover, within this sample there was no correlation between the sociosexual variables and either the frequency of cervical disease or the rate of HPV DNA detection. If it is assumed that all exophytic condylomas and all unequivocal cervical lesions are associated with HPV infection (even though HPV DNA detection rates presently approximate 95% and 90%, respectively[6]), then a minimum estimate of the extent of HPV exposure can be made (Table II). The detection of 31 external condylomas and 94 definite cervical lesions yielded a cumulative frequency for overt disease of 11.5% (Fig. 3). When the 72 women in whom HPV DNA was detected by Southern blot hybridization are added, despite the absence of a definite lower genital tract lesion, the minimal estimate of HPV colonization is 18.6%. Whether the remaining 123 (12.2%) patients with at least one positive morphologic screening test result represent HPV infection is an open question.

Type-specific HPV detection patterns. HPV DNA was detected by low-stringency Southern blot hybridization in 120 (11.8%) of these 1012 women. Type-specific detection rates are enumerated in Table III. Especially noteworthy is the detection of one of the "oncogenic" types in 55 (5.4%) subjects, including HPV 16 in 19 (1.9%), HPV 18 in 14 (1.4%), and HPV 31, 33, 35, 45, 51, 52, or 56 in 22 (2.2%). In contrast, HPVs 6, 11, 42, 43, and 44 were found relatively infrequently in these cervical swabbings. The fact that 43 of 120 (35.8%) positive hybridizations represented as yet uncharacterized genital types is also important. However, in that the vast majority of these unspecified types were detected within either minimal atypia or morphologically normal tissue, it appears that many of these HPVs may have little pathogenic potential (Fig. 4). In contrast to the unspecified group, a much larger proportion of the known HPV types did manifest cervical lesions showing well-developed morphologic evidence of viral cytopathic effect.

Performance of each screening test, used alone. With standard cytologic criteria, wherein any patient showing either a high-grade squamous intraepithelial lesion or a definite low-grade squamous intraepithelial lesion would be recalled for colposcopic workup, Papanicolaou smears detected 12 of the 23 high-grade lesions. Apart from disappointing accuracy (sensitivity 52%, specificity 92%), cytologic screening carried the significant disadvantage of recalling another 76 patients with either low-grade or borderline atypia (only 13 of

Table III. Type-specific HPV DNA detection rates, broken down by cervical (but not vulvar) pathologic condition

	HPV grouping						
Cervical morphology	*HPV 16*	*HPV 18, 45*	*HPV 31, 33, 35, 51 52, 56*	*HPV 6, 11, 42, 43*	*Unclass HPV*	*HPV negative*	*Total*
High-grade	6	3	4	1	2	7	23
Low-grade squamous intraepithelial lesion	5	3	4	11	9	39	71
Equivocal test result	6	2	5	6	15	130	164
Morphologically negative cervix*	2	6	9	4	17	716	754
TOTAL	19	14	22	22	43	892	1012

*Fifteen women had negative cytology, cervicography, and DNA tests, despite having exophytic vulvovaginal condylomas.

which contained some oncogenic HPV type). Attempts to reduce this high recall rate by restricting cutoff criteria to just those women with high-grade atypia led to a calamitous fall in sensitivity (35%). Conversely, attempts to make cytologic testing more sensitive by liberalizing the cutoff point to include women with atypia of uncertain significance led to the recall of 16.5% of patients, for the benefit of detecting just one additional high-grade lesion. Of course, in a real-life situation, some of the 11 patients with high-grade lesions that were missed by this single smear would have been recalled during the cytologic follow-up of equivocal atypia.

Used alone, cervicography and DNA hybridization each detected 14 of the 23 high-grade squamous intraepithelial lesions (61%), and both methods were verified to be valid screening tests when plotted on receiver operating characteristic curves (Fig. 5). However, neither was significantly better nor significantly worse than cytologic testing. Gains in sensitivity generally produced a loss in specificity. For example, combinations of cytologic testing plus hybridization raised sensitivity to 83% but reduced specificity to 88%.

Use of multiple screening tests. The sensitivity of cytologic testing was increased dramatically by the addition of either DNA hybridization or cervicography. However, unless recall was limited to those patients with just a high-grade abnormality by any of the screening tests, the combination of two tests produced an unrealistically high recall rate (20.3% for cytologic testing plus hybridization and 18.7% for cytologic testing plus cervicography). Better performance was obtained by using three tests in an attempt to reliably identify just the high-grade lesions (Fig. 6).

In our search for a solution to this problem, our model system suggested that we should designate a new end point, namely, the presence of either an established lesion or a potential precursor. Recalling those women who had a high-grade morphologic abnormality on either cytologic or cervicographic results irrespective of viral status and patients with minor grade aberrations containing an oncogenic HPV type detected 22 of 23 high-grade lesions (96%), at a cost of recalling only 3.5% of the population (that is, half of those recalled when cytologic testing was used) (Fig. 7).

The greater efficiency of the three-test regimen clearly offers the potential for cost savings (Fig. 8). If monetary values are assigned to the detection and treatment of just the prevalent squamous intraepithelial lesions (i.e., any future incidence of squamous intraepithelial lesions is ignored) ultimate expenditure during the first year of the extended screening program would approximate that of screening with just the Papanicolaou smear (Fig. 9). However, in subsequent years, the population screened by the extended regimen would contain only 4% false-negative results, for which triage and treatment costs would still have to be paid, compared with 48% false-negative results in the traditional cytologic program. Even without factoring in the enormous economic costs of having a proportion of false-negative cases progress to invasive cancer, the extended screening regimen would reduce costs by about one third.

Comment

The incidence of and mortality from cervical cancer have decreased steadily in developed Western countries since the 1950s. In Finland, population-based screening has reduced cervical cancer rates to 5.5 per 100,000 compared with 15.5 per 100,000 in Norway (without a nationwide program).[12, 13] In the United States age-adjusted mortality over the last decade has shown an overall decline of 18%.[14] However, mortality rates among women under age 45 remained stable during this period, while the incidence actually increased in this subset.[15] Moreover, modern screening programs are beset with logistic difficulties. There is considerable lay disillusionment over the high false negativity of Papanicolaou smears. Because of federal regulations limiting the number of slides that can be screened by each

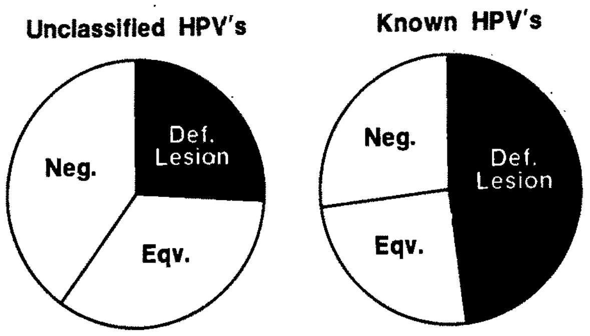

Fig. 4. Pie charts comparing patterns of tissue expression for known versus unspecified HPV types.

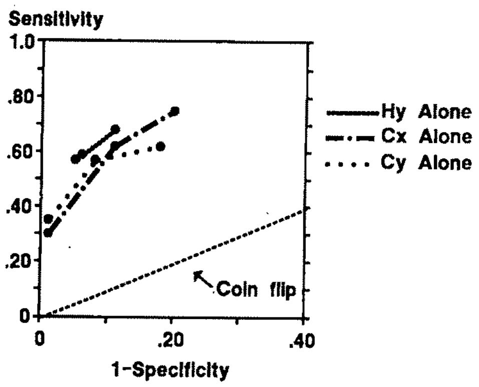

Fig. 5. Receiver operating characteristic curve comparing screening performance of cytologic testing, cervicography, and hybridization, used alone. (Note 2 : 1 transform of Y axis.)

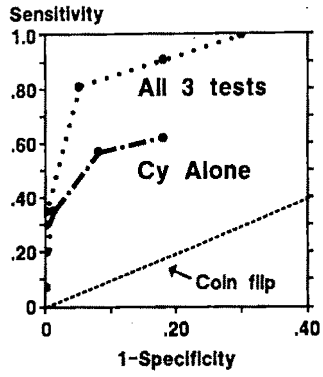

Fig. 6. Receiver operating characteristic curve showing effects of using three screening tests to selectively identify only those patients thought to have just a high-grade lesion. (Note 2 : 1 transform of Y axis.)

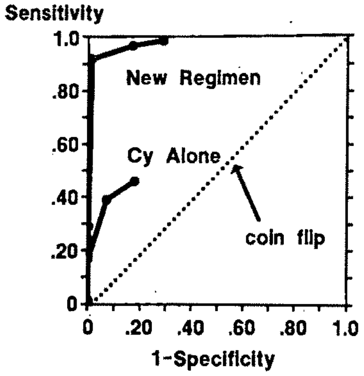

Fig. 7. Receiver operating characteristic curve showing effectiveness of screening for established or potential precursors.

cytotechnician, cytology laboratories now have difficulty in reading their assigned work load. Automated cytologic screening cannot yet fill this deficit. Colposcopic facilities are being overwhelmed by the large number of minor grade abnormalities being reported as "atypical," reflecting both a real increase in the prevalence of sexually transmitted HPV infections and a tendency toward liberalization of cytologic criteria. Maintaining or improving on screening benefits is likely to become increasingly difficult, as the birth cohorts who were teenagers and young adults during the "sexual revolution" approach menopause (the modal age for cervical cancer). For example, a careful analysis of 49 cervical cancers occurring in Rhode Island in 1987 showed that failure to attend for screening accounted for only 15% of cancers in women aged 20 to 39 years, compared with 65% of cancers in the 40 to 69 age group.[16] In contrast, 67% of the younger women had had a negative smear within 3 years of diagnosis, compared with 35% of the older group. Hence there is a need to critically reappraise traditional cervical screening practices, in terms of both what cutoff criteria are most suited to modern-day cytologic screening programs and what contribution might be expected from the incorporation of adjunctive tests.

Analysis of these 1012 women with known disease status suggests that exfoliative cytologic testing cannot be adapted to deliver improved sensitivity. Used as an isolated screening test (rather than as a series of examinations in a coordinated health care program), conventional cytologic criteria yielded a sensitivity of 52% and a specificity of 92%. Detecting 12 of the 23 high-grade squamous intraepithelial lesions in this data base required the recall of 8.7% of the screened population. Receiver operating characteristic curve analysis showed that more restricted cytologic cutoff points would markedly improve specificity but at a cost of a precipitate fall in sensitivity (52% to 35%). Conversely, liberalization of cytologic cutoff points seriously eroded specificity (92% to 84%) for a small gain in sensitivity (52% to 56%). Application of these liberalized criteria in routine clinical practice would have led to the recall of 16.5% of the screened population for the detection of only 13 of 23 known high-grade squamous intraepithelial lesions. Current trends to liberalize cytologic recall criteria, either in response to the introduction of the Bethesda terminology or as a hedge against poten-

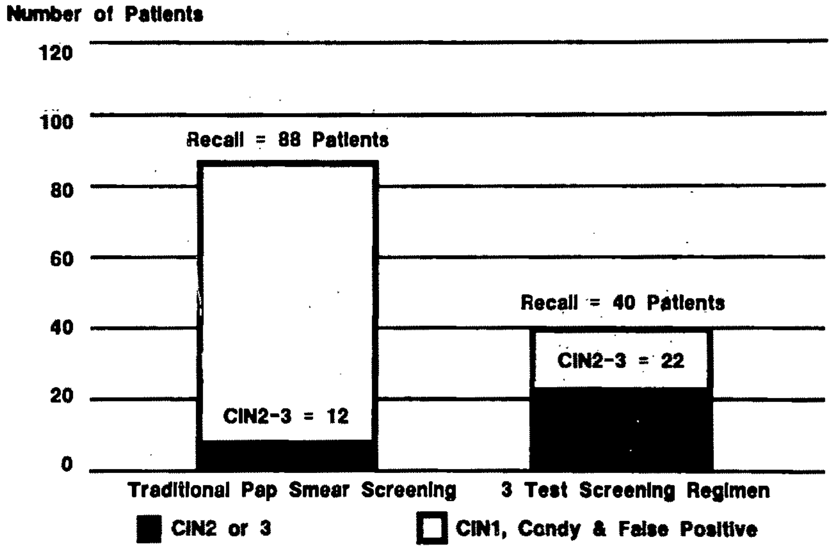

Fig. 8. Relative efficiencies of three-test regimen (40 recalls, detecting 22 cases of cervical intraepithelial neoplasia grade 2 or 3) versus the traditional Papanicolaou smear regimen (88 recalls, detecting 12 cases of cervical intraepithelial neoplasia grade 2 or 3).

Comparison of Projected Costs

CYTOLOGY ALONE (8.7% RECALL = 88 WOMEN)	
Initial screening visit $ ($40 x 1012)	40,480
Screening test cost ($25 x 1012)	25,300
Colposcopy & biopsy ($300 x 88)	26,400
Histology charges ($150 x 88)	13,200
Two colp. & cyto. follow-ups ($200 x 88 x 2)	35,200
	$140,580
Number of high grade SIL's detected	12
Cost per case	$11,715

3 TEST REGIMEN (4.0% RECALL = 40 WOMEN)	
Initial screening visit $ ($40 x 1012)	40,480
3 screening tests ($75 x 1012)	75,900
Colposcopy & biopsy ($300 x 40)	12,000
Histology charges ($150 x 40)	6,000
Two colp. & cyto. follow-ups ($200 x 40 x 2)	16,000
	$150,380
Number of high grade SIL's detected	22
Cost per case	$6,835

Fig. 9. Assigning monetary values shows that the extended screening regimen could produce savings of about 42% for each high-grade squamous intraepithelial lesion detection.

tial litigation, will produce a sharp decline in predictive value and place additional strain on existing diagnostic services. Hence this study suggests that traditional cytologic recall criteria represent the most advantageous compromise between sensitivity and specificity and that improved efficiency of a single screening examination will be achieved only by the addition of adjunctive measures.

Two potential adjunctive methods studied in direct comparison with cytologic testing proved to be independent and valid screening tests. Neither cervicography (sensitivity 61%, specificity 88%) nor DNA hybridization (sensitivity 61%, specificity 94%) was significantly better or significantly worse than exfoliative cytologic testing. Any simple combination of cytologic testing with either cervicography or DNA hybridization did improve sensitivity. However, both combinations tended to erode positive predictive values because of the relatively high incidence of both unsuspected acetowhite cervical epithelium and latent HPV infection in the general population. In contrast, combination of all three screening tests did allow the use of more restrictive criteria, such that one could recall only those patients with a high-grade abnormality by one or another test result, leading to a sensitivity of 83% and a recall cost of 7%. Even so, missing 17% of high-grade squamous intraepithelial lesions at any given screening examination is discomforting and carries an inevitable risk that a proportion of these false-negative cases might progress to invasive cancer during the screening interval.

Optimal sensitivity (96% sensitivity with 4% recall) required selection of a different group of end points; namely, (1) any high-grade cytologic or high-grade cervicographic lesion, irrespective of hybridization result, and (2) any low-grade morphologic atypia in which hybridization detected an oncogenic HPV type. Using this data base as a model system suggested that the additional costs of performing three screening tests could be offset by substantially reduced expenditure on diagnostic triage and empiric therapy.

In interpreting these results, the limitations of this data base must be borne in mind. First, this study was relatively small (1012 patients). Second, there was poor compliance from the STD population, many of whom gave false names and addresses and proved untraceable. Third, initial attempts at cervicography did not reach the proficiency attained in the last three quarters of the study. Finally, the measure of outcome was partly dependent on three cases in which histologic diagnosis was not available. This data base is not justification in itself for altering screening behavior; it simply outlines a provocative model that should be subjected to future testing. Any future decision to expand mass screening for cervical cancer should be supported by evidence from randomized clinical trials.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. JOHN V. KNAUS, Evanston, Illinois. The Papanicolaou smear of cervical cytologic testing is actively undergoing the test of time and science—sensitivity, specificity, statistical analysis, and scrutiny in the literature. HPV is suddenly, and biologically, nearly inseparable from the Papanicolaou smear. Exfoliative cervical cytologic testing, as we have known it, will never be the same.

Dr. Reid and his colleagues pose the relatively high false-negative rate of the Papanicolaou smear as one of the critical issues involving cervical cytologic testing today. They examined cervical cytologic testing coupled with allied intermediate tests in an effort to improve cervical cytologic screening accuracy. The authors' results show neither cervicography nor HPV DNA hybridization alone to be significantly better or worse than cytologic testing alone. The addition of cervicography or HPV DNA hybridization to cytologic testing produced an unrealistically high patient recall rate. Use of all three tests in this model suggested establishing a different end point—the presence of either an established lesion or a potential precursor. This is an important point. The individual host tissue response to HPV exposure is extremely variable. As it should be, histologic confirmation of cytologically suspicious high-grade genital tract intraepithelial neoplasia is the standard on which therapy should be determined. Cost-ineffectiveness will remain until a screening test or tests materialize whose sensitivity and specificity improve the detection rate of clinically significant neoplastic precursors.

The authors offer us a beginning and evoke a series of questions that must be answered in the future. What will be the final resolution of the relatively high false-negative rate for a single screening cervical cytologic smear? Which tests will ultimately improve the failure rate for precursor lesion detection of 10% in patients with cervical cancer who have complied with regular cervical cytologic screening? When will assays with predictive value for reliable HPV detection be available? And finally how will the studies be achieved to reliably predict the spontaneous regression rate of the various HPV type–associated intraepithelial lower genital tract lesions?

Dr. Kenneth L. Noller, Worcester, Massachusetts. The detection of cervical cancer and its precursors always has been one of the primary missions of the gynecologist. With the widespread introduction of cervical cytologic screening in the mid 1950s, many believed that the ideal test for the detection of cervical neoplasia had been developed. However, as with so many of our technologies—continuous electronic fetal monitoring and routine obstetric ultrasonography, to name but two—it is only after widespread use that we begin to understand their limitations.

Unfortunately, few physicians fully understand the scientific basis for disease screening. First, and most important, no screening test ever identifies all cases of a disease. Just as there is never a single cause for any disease, so also is there never one screening method that will detect all individuals at risk for a given illness. The best we can achieve is to identify a subset of the population that is most likely to have or to develop the condition of interest. Indeed, the harder we try to be very specific and to identify all individuals at risk, the more likely we are to falsely assign normal persons to the potentially diseased category. Every screening test will fail to detect some diseased individuals.

Epidemiologists have developed measures for the predictability of screening tests. The four most useful measures are specificity, sensitivity, positive predictive value and negative predictive value. However, to develop a new screening test and to determine these values, it is necessary to know exactly how many individuals in the reference population have the disease of interest by some means other than the test being studied. Unfortunately, that number is unknown in studies of the type performed by Dr. Reid and his associates. The number of individuals with disease in this article was calculated by adding together those individuals found to have disease by the screening procedures under study. While it is difficult to perform a study any better than the way it was conducted by Dr. Reid, it is certainly not fair to apply the terms *specificity* and *sensitivity* to the results because the true number of individuals with disease is not known.

Many investigators are attempting to identify methods to increase the accuracy of Papanicolaou smears. We have a new, federally mandated cytology reporting system (the Bethesda System), a new method of screening (cervicography), and a myriad of ways to look for evidence of HPV infection. Yet all of these innovations have resulted only in causing more confusion. Dr. Reid's article helps to sort through this confusion a little by observing that multiple screening tests for the detection of cervical cancer precursors may more accurately identify those individuals at risk for disease, when compared with any one technique alone. Whereas I am not convinced that the authors have presented any meaningful data proving that their multiple test approach is cost-effective (indeed the comments regarding cost-effectiveness seem to be an afterthought), the results do suggest that it might be useful for us to consider screening individuals with multiple methods.

I do need to make one personal comment. I am tired of reading about, hearing about, and receiving from Papanicolaou smear laboratories, reports of "a few atypical squamous cells." It is time to remember that the purpose of cervical cancer screening is to detect cervical cancer and its precursors, not to diagnose infection. By far, the weight of evidence at the present time suggests that HPV infection and mild dysplasia are not cancer precursors in the vast majority of the patients in whom they are detected. Specifically, a given woman with an HPV infection or mild dysplasia has an almost zero probability that cervical cancer will ever develop, even if no treatment is performed. We have learned, and I hope everyone in this room has learned, that it is futile to attempt to treat asymptomatic HPV infection. In addition, pathologists and cytologists have confessed that they cannot differentiate HPV infection from mild dysplasia; indeed the new Bethesda System equates the two. To his credit, Dr. Reid has clearly identified patients with real disease (cervical intraepithelial neoplasia grades 2 and 3) from those with only minor cytologic and morphologic abnormalities of no significance.

I have only one question for Dr. Reid. Do you really believe that HPV DNA detection kits are clinically useful at the present time? The Centers for Disease Control and most HPV investigators have publically stated that they do not believe that these tests have any clinical relevance at the present time and should remain investigative.

Dr. Eduard Eichner, Cleveland, Ohio. Nobody has really said what I think is the biggest fault of Papanicolaou smears–that it the doctor who obtained them not fixing them properly or getting them stained eventually. In 1947 and 1948 I spent a little time with Dr. Papanicolaou. For several years I did my own tests until Dr. James Reagan came to Cleveland.

We found that there were laboratories that were giving us 100% false-positive or false-negative results, and the technicians were paid by the number of slides they analyzed per hour. They would process with no true vision, and the pathologist himself was not capable of reviewing what the report was. We finally got good pathologists who knew cytology and gave as close to 100% of a true report that we ever had in Cleveland.

Dr. Susan R. Johnson, Iowa City, Iowa. Perhaps I missed this in the presentation, but in the design of the study low-risk and high-risk women were nicely separated. However, I don't recall you saying whether the receiver operating characteristic curves from the two different risk groups were analyzed separately. Maybe the sample sizes were too small to do that. But in a day when insurance companies and others are trying to tell us to reduce Papanicolaou smear screening to every 3 years, these data would help determine if this is sometimes not appropriate. What do you think?

Dr. David V. Foley, Milwaukee, Wisconsin. I couldn't let this opportunity go by without mentioning the Milwaukee opinions. We are, of course, very involved in the cervicography studies and have found this a tremendous asset. I assure you that the money that Dr. Stafl gets from these photographs goes to our university, and we are very proud of that fact.

There is some problem with cervicography though. You have to learn how to use the camera properly and how to interpret the results properly; then I think we will have a definite adjunct to therapy.

One question is are you suggesting that this become a routine procedure in STD clinics and repeating it at yearly intervals? The cost can get out of hand if you do that sort of thing.

Dr. Reid (Closing). Dr. Knaus has asked if we can ever truly abolish the false-negative rate of the Papanicolaou smear. My answer is that I don't think that we can, principally because the Papanicolaou smear was constructed to favor specificity over sensitivity. In a similar vein, Dr. Eichner mentioned that the major contribution to laboratory error is failure by the clinician to adequately fix the glass slide before air drying can occur. Certainly, I agree with Dr. Eichner that if the clinician (or for that matter, the laboratory) does his job poorly, false-negative rates will balloon. However, I stress that the converse is not true. No matter how optimal the technique, false-negative results can never be abolished. For any single test screening regimen, the only realistic alternative to a high false-negative rate is reduced specificity and an unacceptably high false-positive rate. Unfortunately, the introduction of the Bethesda terminology is tending have this effect, thereby overloading colposcopic clinics to near breaking point.

The question was asked as to whether there is a single adjuvant screening test that would reliably abolish the problem of false-negative screenings. If I could look into the future, I would speculate that measuring HPV E6/E7 expression (the viral transforming genes) offers the best prospect of achieving this goal. Such an approach may soon become feasible, either by tests for exon-specific viral messenger ribonucleic acid production or by viral protein analysis. As to which of the presently available adjuvant tests will best detect an occult precursor lesion, I believe that the three-test combination offers the best strategy. If I had to pick just one adjuvant test, then I would choose cervicography in younger populations (where the transformation zone is usually visible on the cervical portio) and viral testing in older populations (where the disease can be wholly within the canal).

Dr. Noller has suggested that our data base does not have a valid "gold standard," against which to calculate sensitivity and specificity. Certainly, if Dr. Noller contends that we should have done a cone biopsy in all of the negative cases, then he is correct in maintaining that we lack such a "gold standard." However, ethics obviously preclude any such approach. If one reviews our methods, it will be clear that we come as close as is practicable to achieving a "gold standard." First, all but three of the patients with significantly positive results underwent colposcopy and biopsy. Second, we believe that patients who had normal results from physical examination, cytology, cervicography, and HPV testing can reasonably be accepted as "true negatives."

With regard to Dr. Noller's question as to what the Virapap offers, I agree that there are too many false-positive results with the kit as presently constituted. However, this is not to belittle the true achievements of this procedure. The Virapap is really a highly successful prototype that has replaced the slow, complex, highly specialized, poorly reproducible Southern blot with a quick, relatively cheap, highly reproducible alternative. Moreover, Virapap can be performed on batched (rather than single) samples, can be performed by a technician (rather than a scientist), and is available in nonspecialized laboratories through the use of a prepackaged kit.

As regards the practical value of Virapap today, this test would perform well as a part of the three-test model system described in our article. However, the mixture of viral probes in the existing Virapap kit is now obsolete and needs to be upgraded in the future. HPV 6/11 should be omitted from this screening kit, because we don't want to recall these patients for triage. Conversely, probes for more recently isolated oncogenic types (such as HPV 45, 51, 52, and 56) need to be included into the upgraded screening "cocktail."

Dr. Johnson asked if we have raw curves for the low- and high-risk populations. The answer is no, partly because only 1.7% satisfied the American Cancer Society's definition of women suited to screening every 3 years. However, the existing data did show a surprising similarity in the age-specific prevalence patterns between the groups recruited from STD and gynecology clinics.

Dr. Foley raised the question of how should we use cervicography. Certainly, both his experience and ours attest to the validity of this method. However, adding cytologic screening plus cervicography ad hoc can exacerbate the false-positive problem by increasing the need to recall a lot of patients with minor cervical lesions, of uncertain progressive potential.

The question of how often to screen a high-risk population is an important one. The data base presented here is only a model and does not allow us to calculate a final recommendation. For the future, diagnostic trials need to be undertaken, to allow quantification of the ideal screening interval.

Trends in the operative management of invasive squamous carcinoma of the vulva at Indiana University, 1974 to 1988

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From January 1974 to March 1988, 150 patients with primary invasive squamous cell carcinoma of the vulva underwent surgery at Indiana University. There has been a trend toward more conservative surgical management of this disease. To determine the impact of this trend on clinical outcome, cases were divided into three groups according to date of operation: group I, 1974 to 1978; group II, 1979 to 1983; and group III, 1984 to 1988. Overall, 80 patients had en bloc radical vulvectomy and groin dissection, 20 had modified radical vulvectomy and bilateral groin dissection through three separate incisions, and 36 had modified radical vulvectomy and unilateral superficial groin dissection. Fourteen patients had other operations. Forty-two patients (27.3%) had radiotherapy in addition to surgery. Among the three groups, there were no differences when mean age, International Federation of Gynecology and Obstetrics stage distribution (1988 system), mean lesion size, mean depth of invasion, or grade distribution were compared. A significant trend toward more conservative surgical therapy was observed. En bloc radical vulvectomy was performed in 77.4% of group I patients, 71.1% of group II patients, and 35.8% of group III patients ($p < 0.001$). Mean days of hospitalization were also reduced significantly. Group I had a mean stay of 30 days, group II had a mean stay of 23 days, and group III had a mean stay of 11 days ($p < 0.001$). Mean operative blood loss (group I, 754.8 ml; group II, 620.0 ml; group III, 393.6 ml; $p = 0.03$), mean units of blood transfused (group I, 1.4 units; group II, 1.3 units; group III, 0.4 units; $p < 0.01$), and mean hours of operating time (group I, 3.7 hours; group II, 3.7 hours; group III, 3.2 hours; $p = 0.02$) were also reduced. (AM J OBSTET GYNECOL 1991;164:1472-81.)

Key words: Squamous carcinoma of the vulva, groin dissection,

Radical vulvectomy with en bloc inguinofemoral lymphadenectomy was first introduced by Bassett[1] in 1912 and has been the standard treatment for invasive squamous carcinoma of the vulva for many years. The prolonged hospitalization and substantial morbidity associated with this operation have inspired many modifications. Taussig[2] suggested doing the operation through "... three incisions, one over each groin and one over the vulva, retaining a bridge of normal skin between them ...," and first Byron et al.[3] and later Hacker et al.[4] adopted this procedure with success. Brunschwig and Brockunier[5] advocated even more conservative therapy for "early" lesions, and other authors have helped to define the limits of such an operation. DiSaia et al.[6] successfully treated 18 patients with modified radical vulvectomy and superficial inguinal lymphadenectomy and prescribed this approach for patients with lesions of <1 cm in diameter and <5 mm in depth. During the last 15 years, these and other surgical modifications have been incorporated in the management of patients with vulvar carcinoma at Indiana University. This study was undertaken to determine what effect evolving operative techniques have had on the treatment of patients with invasive squamous carcinoma of the vulva.

Material and methods

Biopsy specimens confirming the diagnosis of invasive squamous carcinoma were reviewed in all cases by members of the Department of Pathology at Indiana University before surgical therapy.

En bloc radical vulvectomy and inguinofemoral lymphadenectomy were performed as described by Rutledge et al.[7] Primary closure was accomplished in nearly all cases. Radical vulvectomy and bilateral inguinofemoral lymphadenectomy through three incisions were performed as described by Hacker et al.,[4] except that the oblique groin incisions were rarely carried cephalad. The three-incision approach was not used in patients with clinically suspicious inguinal lymph nodes or with primary lesions extensive enough to risk inadequate margins if this technique was used.

Modified radical hemivulvectomy and ipsilateral superficial inguinal lymphadenectomy were used only if

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the primary lesion was ≤ 2 cm in diameter and invaded <5 mm into the underlying stroma. In addition, frozen section was used to evaluate the lymph nodes for metastases. If a midline lesion met these criteria, bilateral groin dissections were performed. The procedure was similar to that described by DiSaia et al.,[6] except that some patients underwent superficial femoral lymphadenectomy as well as inguinal lymphadenectomy. All patients had closed suction drainage of lymphadenectomy sites until daily drainage volume was <30 ml.

No specific attempt was made to determine either the depth of invasion or the adequacy of surgical margins at the time of primary surgery. Generally, a 2 cm margin around the clinically apparent lesion was obtained.

Before November 1977, patients with inguinal or femoral lymph node metastases underwent pelvic lymphadenectomy with postoperative inguinal and pelvic radiotherapy. From that date until July 1984, these patients were placed in a Gynecologic Oncology Group trial in which they were randomized between pelvic lymphadenectomy and radiotherapy. From July 1984, they were treated with postoperative radiotherapy without pelvic node dissection, unless resectable gross pelvic metastases were present.

Records were reviewed and the stage assigned retrospectively according to 1988 International Federation of Gynecology and Obstetrics (FIGO) classification for vulvar carcinoma (Table I). Where follow-up was incomplete, information was obtained by mailings to referring physicians or patients. Histologic evaluation was performed on formalin-fixed, paraffin-embedded 5 μm sections stained with hematoxylin and eosin.

Statistical comparison between groups was performed using the χ^2 test on True Epistat version 3.0 (Epistat Services, Richardson, Tex.). Survival and interval to recurrence was determined by Kaplan-Meier estimate, and intergroup survival comparisons were calculated by the Peto and Peto log-rank test with National Institutes of Health CLINFO software.

Results

From January 1974 to March 1988, 150 patients with primary invasive squamous carcinoma of the vulva underwent definitive surgery at Indiana University Medical Center. For comparison, patients were divided into three groups according to the date of surgery: Group I consisted of 31 patients undergoing surgery in 1974 through 1978, group II included 38 patients having surgery from 1979 through 1983, and group III was composed of 81 patients operated on in 1984 through 1988.

Eighty patients underwent en bloc radical vulvectomy and bilateral groin lymphadenectomy. Twenty patients had a radical vulvectomy and groin dissection performed through three incisions. Thirty-six patients underwent modified radical hemivulvectomy and superficial groin dissection for early lesions; 12 had bilateral groin dissection and 24 had a unilateral procedure. Fourteen patients had other procedures. These include 10 who underwent simple vulvectomy or wide local excision for lesions with ≤ 1 mm of stromal invasion, two who underwent radical vulvectomy without inguinal lymphadenectomy because of anesthetic risk, and one each who had anterior exenteration and laparotomy with paraaortic lymphadenectomy only.

Table I. 1988 FIGO staging classification for vulvar carcinoma

Stage 0	
TIS	Carcinoma in situ
Stage I	
T1N0M0	Tumor confined to vulva and/or perineum, ≤ 2 cm in greatest dimension
Stage II	
T2N0M0	Tumor confined to vulva and/or perineum, >2 cm in greatest dimension
Stage III	
T3N0M0 T3N1M0 T1N1M0 T2N1M0	Tumor of any size with: 1. Adjacent spread to lower urethra and/or vagina or anus, and/or 2. Unilateral regional lymph node metastasis
Stage IVA	
T1N2M0 T2N2M0 T3N2M0 T4, Any N, M0	Tumor invades any of the following: upper urethra, bladder mucosa, rectal mucosa, pelvic bone, and/or bilateral regional node metastasis
Stage IVB	
Any T, any N, M1	Any distant metastasis, including pelvic lymph nodes

Twenty-seven patients received postoperative radiation therapy to the pelvis and groins. Fifteen patients received other radiation therapy. There was no difference in the percentage of patients in each group who received radiotherapy. In group I, nine (29.0%) were treated, and in groups II and III, 12 (31.6%) and 21 (25.9%), respectively, received irradiation.

The age range of patients in this study was 26 to 89 years with a mean of 64. In groups I, II, and III the mean was 69, 68, and 63 years, respectively. Although patients in the third group were younger on the average, there was no significant age difference among the three groups ($p = 0.44$).

In all, 62 patients had FIGO stage I lesions, 44 had FIGO stage II disease, and 28 and 16, respectively, had stage III and IV tumors. Fig. 1 depicts the distribution of FIGO stages in the three groups of patients in this study. Stage I lesions occurred in 38.7%, 36.8%, and 44.4% of cases in sets I through III, respectively. There was no difference in the distribution of FIGO stages among the three groups ($p = 0.34$).

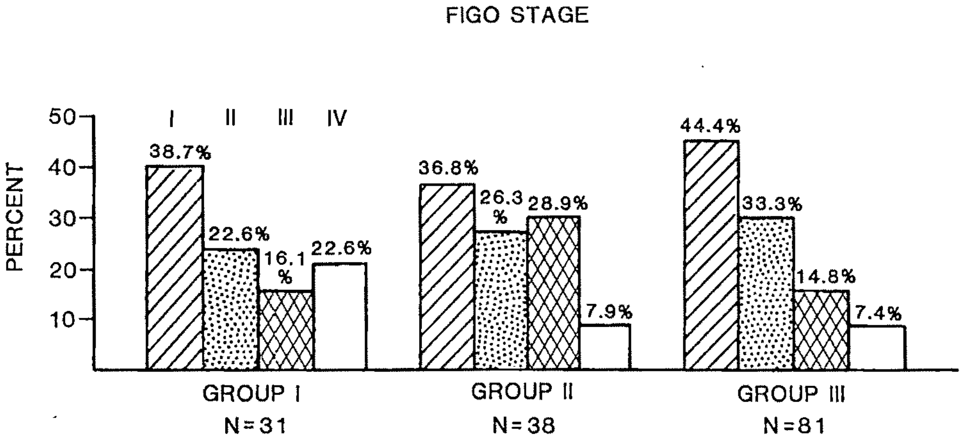

Fig. 1. FIGO stage distribution.

Table II. Recurrences and deaths—groups I and II

Patient	*Age (yr)*	*Stage*	*Recurrence (mo.)*	*Site*	*Therapy*	*Survival (mo.)*	*Status*
Group I							
L.J.	78	II	7.3	Vulva	Radiotherapy	12.2+	No evidence of disease
P.C.	81	II	10.3	Groin	Radiotherapy	10.3	Dead of disease
A.S.	63	III	18.9	Vulva, spine	Radiotherapy	21.8	Dead of disease
Z.A.	76	IV	—	—	—	4.0	Dead of disease
L.C.	54	IV	—	—	—	4.1	Dead of disease
C.W.	65	IV	—	—	—	11.5	Dead of disease
V.D.	83	IV	—	—	—	14	Dead of disease
Group II							
P.B.	89	II	6.9	Vulva	Surgery, radiotherapy	16.1	Dead of disease
M.F.	82	III	10.1	Vulva	Surgery	113.9+	No evidence of disease
C.K.	85	III	—	—	—	4.0	Dead of disease
A.N.	71	IV	—	—	—	1.7	Dead of disease

Overall, 84 patients (56%) had well-differentiated squamous carcinoma. Well-differentiated squamous carcinoma constituted 51.6%, 57.9%, and 56.8% of the populations, respectively, with no difference among the three groups.

Similarly, there was no appreciable difference when tumor size, whether measured by maximum diameter or by surface area, was compared among groups of patients. Diameters ranged from 0.4 to 12.0 cm with a study average of 2.7 cm. For the three subgroups, mean diameters were 2.8, 2.3, and 2.7 cm, respectively. Surface areas ranged from 0.2 to 65.0 cm^2, with means for the study and the three subsets of 9.3, 12.6, 7.3, and 9.1 cm^2, respectively.

The depth of stromal penetration of the tumors averaged 2.8 mm with a range of 0.2 to 8.0 mm with no differences among groups.

Thirty-six patients had metastases to inguinal lymph nodes. Bilateral metastases were present in 13 cases. The percentage of patients with lymph node metastases was greater in groups I and II (29% and 28.9%, respectively) than it was for group III (19.8%), but this difference was not significant.

Follow-up ranged from 1 to 156 months for all patients. Median follow-up was 11 months. There was no difference in median follow-up among the three groups.

The predominant type of operation varied significantly from group to group. Whereas 77.4% of patients in group I underwent en bloc radical vulvectomy and groin dissection, only 35.8% of patients in group III had this procedure. Similarly, no patient in group I had surgery through three incisions, but 19.8% of those in group III had this operation. The selection of operation demonstrated a significant trend from group I through group III ($p < 0.001$, Fig. 2). A significant reduction in operating time was also observed from group I to group III. In the first and second groups, mean time in the operating room was 3.7 hours. This was reduced to 3.2 hours in the third group of patients. Operating time decreased from 4.2 hours in patients undergoing en bloc radical vulvectomy to 3.2 hours and

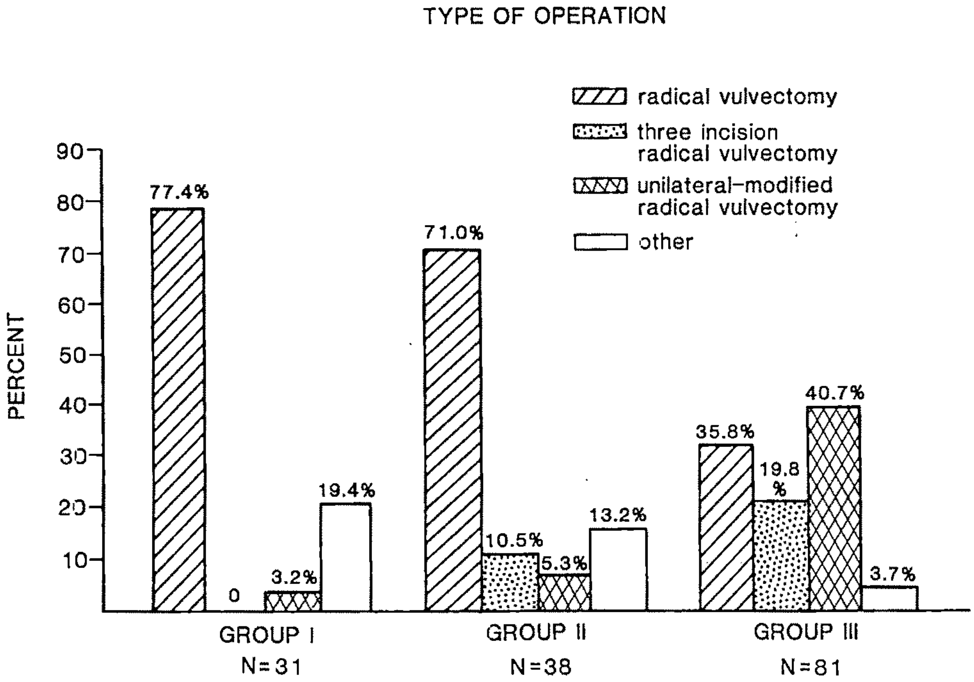

Fig. 2. Distribution of operation.

2.8 hours in those who had three-incision radical vulvectomies and modified hemivulvectomy and ipsilateral groin dissections, respectively. There was no significant difference in days of wound drainage among the three groups. Group I averaged 6.4 days drainage, group II 5.0 days, and group III 7.8 days ($p = 0.6$).

Blood loss and the number of units of blood transfused showed a downward trend, a result of more conservative surgery. In group I, the average blood loss was 754.8 ml (range, 100 to 2500 ml) and an average of 1.4 units (range, 0 to 4 units) was transfused. These averages decreased to 620.0 ml and 1.3 units in group II and 593.6 ml and 0.4 units in group III. The trend in blood loss is significant ($p = 0.03$) as is that for transfusion ($p < 0.01$). The decrease in blood loss and operating time in the three groups is a reflection of less extensive surgery. Patients who underwent radical vulvectomy lost an average of 714.2 ml (range, 150 to 2500 ml), compared with 389.5 ml (range, 140 to 1000 ml) for those who had radical vulvectomies through three incisions and 287.2 ml (range, 50 to 1500 ml) for those who had modified radical vulvectomies and unilateral groin dissections ($p = 0.001$).

Conservative surgery led to a significant decrease in length of hospitalization. The average hospital stay was 30 days (range, 7 to 86 days) in group I, 23 days (range, 3 to 55 days) in group II, and 11 days (range, 2 to 49 days) in group III. This trend is highly significant ($p < 0.001$).

There were a total of 21 recurrences in this study (14.0%). In group I four patients experienced recurrence from 7.0 to 16.0 months after operation, for a crude rate of 12.9%. In group II there were only two recurrences (5.3%) at 8.0 and 10.0 months after operation. The six recurrences in groups I and II were in patients belonging to stage II in three cases, stage III in two cases, and stage IV in one case. All followed en bloc surgery and were equally divided between vulvar and groin failures (Table II). In the third group there were 15 recurrences from 3.0 to 39.0 months after operation for a rate of 18.5%. In group III four patients who had conservative surgery for stage I disease suffered recurrences, all but one with a vulvar component (Table III). Additionally, in five patients with stage II disease and in three patients each with stage III and IV disease recurrent cancer developed. Eighty percent of the recurrences after operation for stage II through IV disease included a vulvar component. Only three of these patients had undergone en bloc surgery. Although the rate of recurrence was greater in group III, this difference failed to reach statistical significance (Fig. 3, $p = 0.077$).

There were eight recurrences involving the vulva or groin among 107 patients who underwent surgery alone. Among 26 patients who received postoperative radiotherapy to the pelvis and groin, two local recurrences were observed. Thus there was no obvious beneficial effect from radiotherapy among these patients.

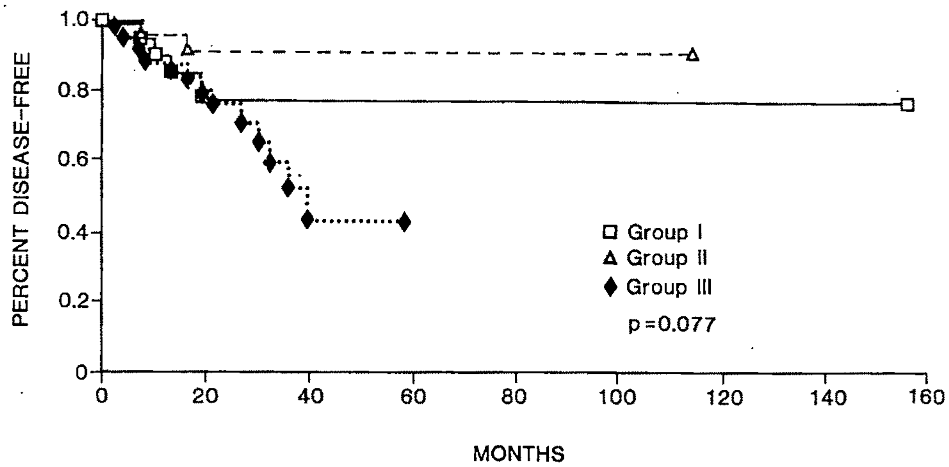

Fig. 3. Relapse-free survival versus group.

Table III. Recurrences and deaths—group III

Patient	*Age (yr)*	*Stage*	*Recurrence (mo.)*	*Site*	*Therapy*	*Survival (mo.)*	*Status*
E.R.	76	I	35.9	Vulva	Surgery	39.9+	No evidence of disease
M.M.	61	I	32.3	Vulva	Surgery	49.6+	No evidence of disease
D.B.	64	I	19.2	Vulva	Surgery	142.1+	No evidence of disease
D.M.	52	I	2.9	Vulva, groin	Surgery, radiotherapy	7.7	Dead of disease
D.R.	75	II	35.9	Vulva	Surgery	39.9+	No evidence of disease
V.A.	86	II	30.0	Vulva	Radiotherapy	30.0+	No evidence of disease
A.P.	72	II	21.0	Groin, lung	Chemotherapy	28.0+	Alive with disease
L.L.	72	II	24.0	Vulva	Surgery, radiotherapy	28.0+	No evidence of disease
D.V.	51	II	16.4	Groin	Chemotherapy	20.3	Dead of disease
E.T.	75	III	4.4	Groin	Radiotherapy	6.2+	Alive with disease
M.H.	68	III	4.3	Vulva	Chemotherapy	103	Dead of disease
M.P.	66	III	6.4	Vulva, groin	Chemotherapy	6.6	Dead of disease
H.H.	60	IV	2.6	Vulva, groin	Chemotherapy	5.6	Dead of disease
S.B.	34	IV	8.0	Vulva	Chemotherapy	8.0	Dead of disease
L.B.	83	IV	6.7	Vulva	Chemotherapy	8.0	Dead of disease

It should be noted that these numbers are too small for appropriate statistical analysis.

Eleven patients with recurrent cancer died of disease and two are alive with evidence of persistent carcinoma. In addition, there were five deaths among patients in groups I and II who were never rendered disease-free after diagnosis. The cancer-related mortality rate for this series of 150 patients is thus 10.7%. Crude survival rates among the three groups are 80.6%, 92.1%, and 91.3%. There were no statistical differences in survival rates among the three groups in this study ($p = 0.24$, Fig. 4).

Surgical complications occurred in 42.8% of cases and are listed in Table IV. In group I 55.1% had surgical complications, compared with 33.9% in group II and 41.5% in group III. There was no difference in complication rates among the three groups. However, the rate of surgical complications increased with increasing age. A surgical complication developed in only 16.7% of patients <30 years old. From ages 30 through 60 years, 33.7% had surgical complications, and over 60 years, 46.2% had surgical complications. Surgical complications decreased in the less extensive operations (Table V).

Comment

Bassett introduced the concept of en bloc radical vulvectomy and inguinofemoral lymphadenectomy for the treatment of carcinoma of the clitoris in 1912. Largely though the efforts of Stanley Way in England and Frederick Taussig in the United States, Bassett's operation became the standard of therapy in the treatment of vulvar malignancies. Both of these authors provided convincing arguments supporting extensive surgery. Plentyl and Friedman[8] summarized a number of surgical series and observed that survival rates improved from 30.8% with radical vulvectomy to 32.5% for radical vulvectomy with radiotherapy and from 55.7% with radical vulvectomy and groin lymphadenectomy to 63.2% when pelvic lymphadenectomy was

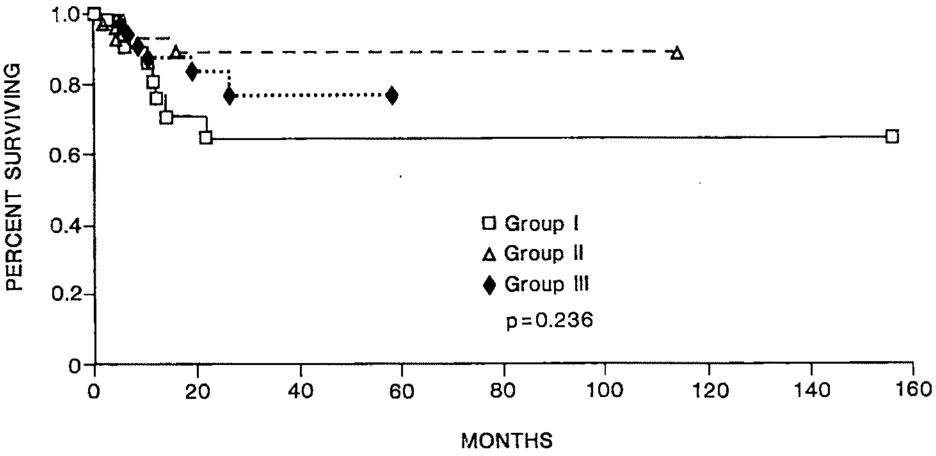

Fig. 4. Survival versus group.

added. Despite efforts to reduce surgical morbidity, such extensive operations are associated with wound complication rates that approach 60%.[9] Other problems such as chronic lymphedema and cellulitis also complicate these procedures.

Although Taussig suggested that radical vulvectomy and inguinal lymphadenectomy be performed through three separate incisions as early as 1940,[2] this concept was not well recognized in the modern surgical era until the publication by Byron et al.[3] in 1962. They advocated separate vertical incisions in the groins, sartorius muscle transposition, and postoperative suction catheter drainage. Ballon and Lamb[10] subsequently treated 18 patients with a similar technique. Most of these patients had primary healing of the groin incisions, and total hospitalization averaged 26 days.

In 1981 Hacker et al.[4] reported a series of 100 patients who underwent radical vulvectomy with the use of three incisions. The incidence of major wound breakdown was only 14%, and 38% of the patients in the series had primary healing of all wounds. Mean hospitalization was 19 days. Recurrent cancer developed in 16 patients. No isolated recurrence developed in the bridge of skin preserved between the groin and vulvar incisions in any patient, but skin-bridge recurrences developed in two in association with distant metastases. Each had positive inguinal lymph nodes.

The early definition and subsequent acceptance of microinvasive carcinoma of the cervix as a clinical entity has inspired a search for a "microinvasive" carcinoma of the vulva. Defining an early vulvar cancer should allow the performance of a conservative vulvar operation. Brunschwig and Brockunier[5] described a small group of patients with limited vulvar lesions who, for medical reasons, underwent local excision or modified vulvectomy. The cure rate in this group of patients was 66%; increasing lesion size was associated with greater risk of failure. In 1971, Franklin and Rutledge[11] reported inguinal metastases in only one of 18 patients with primary lesions measuring <1 cm in diameter and in 15% of 48 patients with lesions <2 cm in diameter. They noted no inguinal metastases in patients with invasive carcinoma arising in areas of carcinoma in situ if stromal invasion was <5 mm. In 1979 DiSaia et al.[6]

Table IV. Surgical complications in 187 patients with invasive vulvar carcinoma

Surgical complication	*No. of patients*
Wound separation	40
Lymphocyst	23
Wound infection-necrosis	14
Lymphedema	10
Hemorrhage-hematoma	7
Cellulitis	7
Lymphangitis	4
Osteomyelitis	3
Vaginal stenosis	2
Vulvar scar (requiring revision)	1
Vaginal fistula	1
Anal stricture	1

Table V. Type of operation compared with surgical complications in 187 patients with invasive vulvar carcinoma

Type of operation	*No. of patients*	*Surgical complications (%)*
Radical vulvectomy	100	56.0
Modified radical vulvectomy with bilateral groin dissection	22	54.5
Modified radical vulvectomy with unilateral groin dissection	39	23.1
Other	26	11.5
TOTAL	187	

reported a series of patient with squamous lesions confined to the vulva or perineum ≤ 1 cm in diameter with focal invasion of <5 mm. Surgery consisted of bilateral superficial inguinal lymphadenectomy and modified radical vulvectomy or hemivulvectomy. Only superficial inguinal and femoral lymph nodes were removed, the cribriform fascia being preserved intact. This approach does not involve the skeletonization of the femoral vessels, which lie below the cribriform fascia. Frozen section of the specimen was obtained and further lymph node surgery was not done in the absence of metastases in these superficial "sentinel" lymph nodes. Wide vulvar excision included a 3 cm margin of normal skin or mucosa surrounding the lesion, as well as adequate subcutaneous tissue, usually to the level of Colles' fascia. Eighteen patients so treated were doing well at 7 to 74 months (mean, 32 months) after operation. Two for whom this therapy was intended had metastases in the sentinel lymph nodes and were treated with standard radical vulvectomy with inguinal and ipsilateral pelvic lymphadenectomy.

The Gynecologic Oncology Group initiated a study in 1983 that involves modified radical hemivulvectomy and ipsilateral superficial inguinal lymphadenectomy with frozen section. Eligible patients are limited to FIGO stage I (tumor diameter <2 cm, clinically negative groin nodes), maximum tumor thickness of 5 mm, no vascular involvement, and surgical margins of 2 cm. Deep surgical margins of 1 cm are required. The inguinal dissection is essentially as described by DiSaia et al. If frozen section examination of the excised nodes reveals metastases, bilateral inguinofemoral lymphadenectomy in the traditional method is performed, and the patient may be a candidate for radiotherapy.

In this series the prescripts of Hacker and DiSaia were used in the treatment of patients with vulvar cancer. This retrospective series confirms the advantages of limited surgery: less time in the operating room, reduced blood loss and associated transfusion, and shortened hospital stay. The cost of these advantages may be an increased risk of local vulvar recurrence. Although such recurrences are usually amenable to surgical treatment or radiotherapy, they may be fatal. An increase in risk cannot be confirmed statistically in this series, but the observed trend is of concern.

The risk of local recurrence after conservative therapy has been reported by others. Burke et al.[12] observed a 10% rate of new or recurrent vulvar disease in a group of 32 patients with T1 or T2 lesions and clinically negative groin nodes managed by radical wide excision and selective lymphadenectomy. Heaps et al.[13] evaluated surgicopathologic features of vulvar cancer to predict recurrent disease. In their study the status of the surgical margin was the strongest indicator of risk for local recurrence. Six of seven patients with positive surgical margins and 21 of 44 patients (47.7%) with margins of <8 mm developed recurrent disease. In contrast, there were no local failures in 91 patients with free margins ≥ 8 mm. A clinicopathological evaluation of treatment failures in the present series is under way.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. JOHN M. MALONE, JR., Detroit, Michigan. There are several criteria that I use in judging the merit of an article. Does it present new information or does it merely confirm what we already know? Are the design and analysis valid? Does it open new areas for investigation? And, finally, does it cause me to alter the way I practice? Dr. Sutton's timely study fulfills some of these criteria but not others.

This article reviews trends in the operative management of invasive vulvar cancer over 14 years at Indiana University and represents one of the first large, single-institution, retrospective studies, assessing the impact of less radical vulvar surgery on morbidity and survival.

The 150 patients are broken into three groups, covering roughly 5 years each. These groups are statistically similar in regard to mean age, stage, depth of penetration, and cellular differentiation. Over time, there was a trend toward less radical surgery. Of the group I patients, 77.4% underwent an en bloc radical vulvectomy and bilateral groin lymphadenectomy, whereas only 19.8% of the group III patients underwent this procedure, $p < 0.001$.

The authors detail the treatments rendered and their effect on measurable outcomes such as mean length of operating time and hospitalization, mean blood loss, and complication rates. Not surprisingly, these parameters all improved with time and fewer radical procedures. As far as effect on survival, the authors mentioned that no statistical difference was seen, $p = 0.77$. They quickly pointed out that even though no difference was seen, a trend was noted.

Recent trends in the therapy of vulvar cancer have been much like those in breast cancer; specifically, fewer radical procedures and more individualization of treatment. Intuitively, this is attractive. The central question, however, is in the quest for an improved quality of life. Have we sacrificed cures, and have we altered survival rates? The authors attempted to answer this question and did so partially; however, many questions were left unanswered and others were generated.

Some unanswered questions are as follows:

1. Mention is made of the use of adjunctive radiation therapy, yet no results are given. Does it help?
2. Mention is made of doing the lymphadenectomy through separate skin incisions. In light of recent reports of recurrence in the skin bridge, did the authors notice any recurrences in this area?[1]
3. As a practical matter, how does the operating surgeon ascertain whether there is ≥ 5 mm of invasion at the time of surgery?
4. Were the specimen margins checked for adequacy at the time of surgery? If not, does this account for any of the recurrences or are local recurrences evidence of the multifocal nature of this disease?
5. Most important, and the one question that I am interested in hearing an answer for today, is do the authors think that the trend toward less radical surgery is safe, or should we be going back to the more comprehensive and radical procedures first developed for this disease?

Finally, in the long run, the balance between care and quality of life is a delicate one and one we face every day. Perhaps the most valuable service that we can provide our patients is to understand the ramifications of treatment, explain them to the patient, and let her decide.

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Dr. Robert D. Hilgers, Springfield, Illinois. This study analyzes 150 cases of squamous cell carcinoma of the vulva treated at The University of Indiana over a 14-year period from 1974 to 1988. The study design is based on three time periods set apart by the dates of surgery. Their results show that a change in the selection of surgical treatment occurred, first using the traditional en bloc radical vulvectomy and groin and pelvic lymphadenectomy and evolving to a more conservative modified radical vulvectomy and superficial node dissection for early carcinoma of the vulva and radiation therapy for treatment of positive groin nodes. Significant differences were noted not only in the selection of the type of operation but also in amount of blood loss, number of transfusions, and average hospital time as the operative procedure became more progressively conservative.

As a result the standard surgical treatment of squamous cell carcinoma of the vulva by radical vulvectomy and groin dissection has been replaced by more individualized treatment, occasionally preserving anatomic structure and function, without losing survival advantage in both early and advanced disease. What constitutes conservative operative management of the vulva and groin nodes and retaining a skin bridge, however, is not universally defined and agreed on, and the core of this presentation, i.e., the three major surgical procedures, as well as the radical wide local excision, may provide further clarification in each of these areas.

The first of these involves the vulva and the limits that can be safely applied without adding risk to the patient by preserving certain anatomic structures removed in the "type ordinaire" radical vulvectomy. In this regard, I wish to ask Dr. Sutton and his colleagues what is meant by a modified radical vulvectomy. The authors state that they had no vulvar recurrences in patients treated by radical vulvectomy and four vulvar recurrences in patients treated by modified radical vulvectomy and superficial inguinal lymphadenectomy in stage I disease. Is there any advantage of performing a modified radical vulvectomy or a radical wide local excision as opposed to a radical vulvectomy, and is the recurrence rate the same for a radical wide local excision or a radical hemivulvectomy as it is for a radical vulvectomy?

Second, the authors observed that the depth of stromal invasion ranged from 0.4 to 8 mm. Did the frequency of groin node metastasis increase with the depth of stromal invasion? Since the superficial inguinal nodes act as the first echelon of lymphatic spread in early squamous cell carcinoma of the vulva, are the superficial inguinal nodes sentinel nodes for predicting deeper nodal metastasis? Did the authors observe in any of their cases an instance where the superficial inguinal nodes were negative and the deep femoral nodes were positive for metastatic disease? Do they feel that there are different indicators for performing a superficial inguinal lymphadenectomy and an inguinal-femoral lymphadenectomy? Ordinarily, a difference in survival exists between patients with negative groin nodes and those with positive groin nodes. Did the authors notice any difference between these two subsets in their series?

Finally, what justifies leaving a skin bridge between the inguinal and the vulvar incisions, and what appears to be a safe recommendation for preserving the skin bridge in these patients?

Dr. James G. Blythe, St. Louis, Missouri. I've been watching in the literature the conservative movement in this field, and, if I understood your article correctly, my conclusion would be that using survival as an end point, as I move toward more conservative therapy it would remain the same.

However, using increased costs, it seems today that conservative therapy increases the recurrences and decreases the quality of care. Therefore my conclusion today would be that I had better look at conservative therapy very, very carefully in these patients and move slowly away from the radical surgery. I did not hear your conclusions. Could you share those, please?

Dr. Eduard Eichner, Cleveland, Ohio. A similar article was presented at the District 5 American College of Obstetricians and Gynecologists meeting in Toronto last week, in which the questions arose as to what was radical surgery, how you define it, and what you do. Since I had done pelvic lymphatic studies in the 1950s and reported them, I was asked to discuss this.

When we did our original vulvar studies, we found, with the usual dye studies, that in all our patients the femoral and inguinal lymphatic nodes were never involved because our patients were lying flat and motionless during the injection period.

We then followed a small injection of the dye as it went over the symphysis into the pelvis to the iliac area and followed the various pelvic lymphatic channels. We then put a small weight (½ to 1 ounce) bilaterally at the angle of the symphysis and then repeated the study on these patients; the dye never went over the symphysis. It went laterally through the femoral area first and then to the inguinal area, as well as the lateral thigh nodes; the only time dye that reached the iliacs was when the injection was made in the perirectal space at the inferior portion of the vulva.

Therefore at that time we started doing a modified Pfannenstiel incision and taking out internal nodes first when performing a vulvectomy. If Cloquet nodes were involved, it was said that the infection had gone deep into the pelvis, and these women all underwent a radical full vulvectomy.

If the femoral nodes were minimally involved or not involved, the incision was then extended to the superficial inguinals. If they were involved, radical surgery was done; if they were not involved, we just did a simple vulvectomy.

Dr. Karl C. Podratz, Rochester, Minnesota. Dr. Sutton, I you addressed the frequency of vulvar recurrence. I was wondering if you might share with us the site of these recurrences, that is, the central versus the lateral resected margins in the area that was resected. And also would you address whether there were contralateral recurrences in sites that were not operated on and the frequency with which you had recurrences in your cutaneous bridge?

Dr. Richard Reid, Southfield, Michigan. Again, I would like to ask Dr. Sutton about the vulvar recurrences. Was there a pattern to them? What were the preoperative surveillance methods used to detect insipid recurrence? Were any of them predictable preoperatively?

President Malinak, Houston, Texas. I would like to ask Dr. Sutton if, over the 14-year period, the differences in the intraoperative methods might have an influence on the difference in morbidity, such as more use of electrosurgical methods, the use of prophylactic antibiotics and that sort of thing?

Dr. Sutton (Closing). First of all, there obviously were changes in techniques over the years, and this study was naive to those changes and just looked at the time blocks. I am certain some of those techniques changed our operative parameters.

In the early part of the study, Dr. Ehrlich did most cases singlehandedly, and in later years he had a little bit of help. I think the number of people involved in the surgery probably affected its duration.

I agree with Dr. Blythe that we need to look critically at the conservative management of vulvar cancer. Not only our series but also Gynecologic Oncology Group series of conservative treatment have disclosed a number of recurrences. We noticed recurrences in the contralateral vulva. I think it really is important to consider the presence of vulvar dystrophy when the operation is planned. There were patients in this series who had a modified hemivulvectomy and had dystrophy in the remaining vulva. I think that that type of operation probably is not justified. I think if there is dystrophy in the contralateral vulva, a complete vulvectomy is indicated.

Dr. Podratz, we did not specifically look at sites of recurrences in terms of medial or lateral. We did look at vulva versus groin. We would have to go back to look at that data to get that information.

Dr. Reid, we had about four out of 36 patients in the third group who had recurrences after surgery for stage I lesions. All four of those patients had a vulvar component. One patient also had relapse in the groin. Interestingly enough, this patient had negative superficial inguinal lymph nodes at the time of operation. We didn't do many femoral node dissections, and when they were done, we didn't take pains to separate the femoral and inguinal specimens. Ideally, a study should be done that looks at that issue critically.

We have accepted the idea of sentinel nodes on the basis of a few studies of patients who did not experience relapse. We don't know that these are sentinel nodes, and I think it would be important to look at the superficial inguinal lymph nodes and the femoral lymph nodes separately.

Regarding the types of surgery, basically we took "wide local or wide radical incision" or "modified radical vulvectomy" to indicate that less than the whole vulva was excised. A radical vulvectomy was performed through three incisions or by en bloc technique, starting laterally at the labial-crural folds and removing the entire vulva.

Did we check the margins at the time of surgery? No, we did not. We attempted to get 2 to 3 cm of normal tissue at the margin of the specimen. I am not aware of any samples in the series that had positive margins at the initial operation.

The depth of invasion was determined on the preoperative biopsy specimen. We decided whether the patient could undergo a modified vulvectomy on the basis of this information. The final depth of invasion was determined on the pathologic specimen. We didn't experience skin bridge recurrences. Generally, if there are palpable or suspicious lymph nodes in the groin, we don't leave a skin bridge; if the lymph nodes are clinically negative, a skin bridge might be left.

I believe the recurrences that have been reported have been in patients who had palpable nodes in the groin.

Did radiation therapy help? We don't know. We didn't separate out those patients specifically, and that is obviously something we need to do.

The fetal concentrating index as a gestational age–independent measure of placental dysfunction in intrauterine growth retardation

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In previous work we have shown, using cordocentesis to obtain fetal blood, that fetuses with intrauterine growth retardation are hypoxic and suffer from in utero starvation of nutrients. In this study we have developed gestational age curves for fetal blood amino acids and carnitine that now allow the development of a new parameter, the fetal concentrating index, which is the numeric mean of the fetal/maternal ratio of six essential and nonessential concentrated amino acids. Our data have shown that this index does not vary with gestation in either normal pregnancies (1.83 ± 0.42, mean ± SD) or pregnancies with intrauterine growth retardation (1.46 ± 0.38), but the index is markedly reduced in intrauterine growth retardation ($p < 0.001$). These results suggest that, because cordocentesis has become very safe in experienced hands, cordocentesis to obtain the fetal concentrating index might ethically be obtained in cases of fetuses at risk for intrauterine growth retardation, to devise strategies for intervention before the onset of severe hypoxia and morphometric changes. (AM J OBSTET GYNECOL 1991;164:1481-90.)

Key words: Fetal concentrating index, intrauterine growth retardation, amino acids, carnitine

In previous work we and others have measured maternal and fetal plasma amino acid concentrations.[1-3] These results have indicated that some amino acids, especially the essential ones, are concentrated in the human fetal circulation, whereas others are not.[3] The concentrating process requires energy, and causes of impaired energy production, e.g., starvation and placental insufficiency, reduce the capacity of the fetus to concentrate essential amino acids, such as valine.[1, 5] We specifically investigated the relationship of maternal and fetal amino acids in appropriate-for-gestational age

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Table I. Plasma amino acid and carnitine concentrations in normal control fetuses in different gestational age groups

	Gestational age		
	16-24 wk (n = 38) (μmol/L)	*25-32 wk (n = 25) (μmol/L)*	*33-36 wk (n = 13) (μmol/L)*
Taurine	119 ± 36 (44-230)	113 ± 42 (75-256)	117 ± 49 (66-221)
Urea	2530 ± 732 (1050-3870)	2457 ± 622 (1394-4112)	2467 ± 705 (1034-3431)
Aspartic acid	10 ± 5 (trace-25)	16 ± 20 (4-82)	11 ± 7 (3-24)
Threonine	230 ± 45 (149-338)	271 ± 61 (161-418)	260 ± 40 (176-302)
Serine	126 ± 25 (84-176)	138 ± 38 (63-217)	130 ± 16 (93-148)
Asparagine	61 ± 24 (7-160)	108 ± 50 (39-203)	111 ± 41 (41-173)
Glutamine	750 ± 287 (126-1550)	621 ± 227 (206-1143)	474 ± 230 (133-1038)
Proline	196 ± 56 (90-396)	203 ± 58 (89-363)	182 ± 32 (136-260)
Glycine	163 ± 49 (88-376)	169 ± 46 (86-353)	177 ± 26 (125-212)
Alanine	293 ± 112 (142-726)	358 ± 154 (146-827)	284 ± 90 (135-498)
Citrulline	16 ± 6 (5-40)	13 ± 5 (6-22)	13 ± 5 (8-27)
Valine	226 ± 48 (138-355)	208 ± 45 (127-286)	200 ± 35 (136-280)
Methionine	32 ± 10 (15-61)	33 ± 10 (19-62)	30 ± 5 (22-39)
Isoleucine	65 ± 17 (32-107)	58 ± 18 (18-78)	59 ± 9 (41-72)
Leucine	110 ± 25 (42-166)	104 ± 26 (62-151)	101 ± 15 (73-118)
Tyrosine	63 ± 17 (35-107)	70 ± 17 (42-103)	72 ± 16 (49-100)
Phenylalanine	64 ± 16 (25-104)	71 ± 14 (55-121)	72 ± 11 (58-93)
Ammonia	98 ± 38 (29-183)	97 ± 58 (30-286)	96 ± 53 (30-185)
Ornithine	122 ± 41 (54-244)	109 ± 36 (51-197)	100 ± 28 (64-156)
Lysine	351 ± 66 (248-485)	347 ± 65 (251-468)	312 ± 61 (163-397)
Histidine	111 ± 27 (59-198)	109 ± 17 (79-137)	112 ± 14 (94-142)
Arginine	80 ± 27 (23-122)	88 ± 31 (31-145)	94 ± 28 (38-136)
Carnitine			
Free	20 ± 7 (7-35) (*n* = 19)	18 ± 9 (5-36) (*n* = 17)	19 ± 8 (10-33) (*n* = 12)
Total	37 ± 15 (18-68) (*n* = 9)	36 ± 12 (21-57) (*n* = 7)	31 ± 9 (20-42) (*n* = 6)

Values are mean ± SD with range shown in parentheses.

(AGA) and small-for-gestational-age (SGA) fetuses and found differences in the amino acid profiles between these groups.[3] The plasma fetal/maternal ratio of a compound indicates whether it is concentrated in the fetal circulation. A substance that is not concentrated has a ratio of approximately 1.0; a highly concentrated substance exhibits a ratio of 2.0 to 3.0. In SGA fetuses the ratio of essential, but not nonessential, amino acids correlated inversely with the changes in umbilical venous Po_2 of fetuses.[1]

Unfortunately, the gestational age variation in the fetal/maternal ratio differs markedly for every amino acid. Moreover, individual amino acids, or separating the essential ones from the nonessential ones, did not by themselves provide a means to prospectively assess impending growth retardation. Therefore we have extended our original observations to include carnitine, which is integral to mitochondrial fatty acid oxygenation and have developed a coordinated parameter, i.e., the fetal concentrating index, to describe quantitatively the ability of the placenta to extract circulating maternal amino acids. This index provides a more powerful measure of fetal concentrating ability than that offered by any single amino acid's fetal/maternal ratio.

Methods

Umbilical venous blood was obtained by cordocentesis at 16 to 36 weeks' gestation from 62 AGA fetuses and 31 SGA fetuses. In all cases gestational age was determined by Nägele's rule and confirmed by an ultrasonographic scan in early pregnancy. In both groups maternal blood was sampled from an antecubital vein immediately before cordocentesis. All the mothers were healthy, had negative screening results for antinuclear factor, and did not show serologic evidence of recent toxoplasmosis, rubella, cytomegalovirus, or syphilis infection. For 75 control pregnancies the indications were: prenatal diagnosis of blood disorders (*n* = 6), fetal infection (*n* = 3), α-antitrypsin disease (*n* = 1), elective termination (*n* = 4), karyotyping for amniotic fluid culture failure (*n* = 4), amniotic fluid mosaicism (*n* = 4), late booking (*n* = 4), and anatomic defects (*n* = 40). Patients with encephaloceles, hydrops, or chromosomal defects that could potentially alter amino acids were excluded from the control group.

Of the control pregnancies studied, 16 were terminated, and four were lost to follow-up. The remaining 55 were delivered at 34 to 40 weeks' gestation. Birth weights, available for 52 of these, were in every

Table II. Fetal/maternal ratios of plasma amino acid and carnitine concentrations in normal control pregnancies of different gestational age groups

	Gestational age		
	16-24 wk (n = 37) (*μmol/L*)	*25-32 wk (n = 25)* (*μmol/L*)	*33-36 wk (n = 13)* (*μmol/L*)
Taurine	1.86 ± 0.82 (0.37-3.74)	2.09 ± 0.81 (0.50-3.58)	1.54 ± 0.59 (0.69-2.75)
Urea	1.18 ± 0.26 (0.68-2.02)	1.02 ± 0.15 (0.68-1.36)	0.97 ± 0.13 (0.69-1.14)
Aspartic acid	0.98 ± 0.48 (0.40-2.50)	1.06 ± 0.84 (0.11-4.55)	0.68 ± 0.40 (0.25-1.75)
Threonine	1.71 ± 0.27 (1.09-2.45)	1.67 ± 0.27 (1.17-2.15)	1.50 ± 0.25 (0.98-1.88)
Serine	1.41 ± 0.35 (0.78-2.27)	1.41 ± 0.35 (0.63-1.89)	1.33 ± 0.35 (0.82-2.13)
Asparagine	1.08 ± 0.33 (0.22-2.11)	1.05 ± 0.26 (0.28-1.47)	1.01 ± 0.21 (0.66-1.52)
Glutamine	1.14 ± 0.39 (0.26-2.08)	1.09 ± 0.20 (0.78-1.56)	0.90 ± 0.33 (0.22-1.32)
Proline	1.57 ± 0.48 (0.61-2.68)	1.35 ± 0.27 (0.79-2.12)	1.14 ± 0.21 (0.92-1.58)
Glycine	1.28 ± 0.36 (0.82-2.67)	1.22 ± 0.27 (0.61-1.70)	1.43 ± 0.37 (0.90-2.23)
Alanine	1.37 ± 0.29 (0.83-2.32)	1.38 ± 0.31 (0.78-2.17)	1.04 ± 0.32 (0.59-1.71)
Valine	1.64 ± 0.31 (0.77-2.22)	1.60 ± 0.31 (1.13-2.07)	1.48 ± 0.30 (1.02-1.98)
Methionine	1.84 ± 0.70 (0.75-3.78)	1.72 ± 0.52 (1.04-2.95)	1.46 ± 0.47 (1.00-2.78)
Isoleucine	1.46 ± 0.36 (0.85-2.17)	1.32 ± 0.39 (0.49-1.97)	1.44 ± 0.33 (0.89-1.97)
Leucine	1.32 ± 0.41 (0.56-2.22)	1.30 ± 0.41 (0.64-2.02)	1.34 ± 0.38 (0.78-1.85)
Tyrosine	2.08 ± 0.42 (1.21-2.83)	1.84 ± 0.33 (1.10-2.47)	1.76 ± 0.30 (1.19-2.49)
Phenylalanine	1.59 ± 0.45 (0.69-2.85)	1.66 ± 0.34 (1.10-2.44)	1.55 ± 0.38 (0.93-2.38)
Ammonia	1.53 ± 0.48 (0.80-3.03)	1.54 ± 0.65 (0.45-3.13)	1.65 ± 0.64 (0.82-2.83)
Ornithine	2.46 ± 0.66 (1.26-3.76)	2.39 ± 0.57 (1.10-3.67)	2.49 ± 0.88 (0.97-4.14)
Lysine	2.41 ± 0.41 (1.41-3.15)	2.48 ± 0.63 (0.94-3.56)	2.45 ± 0.83 (1.08-4.33)
Histidine	1.12 ± 0.36 (0.37-2.05)	1.20 ± 0.34 (0.40-1.80)	1.17 ± 0.28 (0.69-1.64)
Arginine	1.64 ± 0.89 (0.49-5.50)	2.12 ± 1.35 (0.53-7.11)	2.30 ± 1.17 (0.64-3.88)
Carnitine			
Free	0.82 ± 0.39 (0.37-1.59) (*n* = 17)	0.64 ± 0.25 (0.28-1.12) (*n* = 16)	0.91 ± 0.51 (0.43-2.27) (*n* = 12)
Total	1.04 ± 0.34 (0.56-1.39) (*n* = 7)	0.85 ± 0.17 (0.65-1.09) (*n* = 6)	0.92 ± 0.25 (0.50-1.24) (*n* = 6)

Values are mean ±SD with range shown in parentheses.

case greater than the 5th percentile for gestational age.

The 38 patients in the SGA group were referred for fetal karyotyping and blood gas analysis because of ultrasonographic evidence of severe intrauterine growth retardation (IUGR). The fetal abdominal circumference was <25th percentile of our reference range, which was constructed from the study of 1610 normal pregnancies.[6,7] Three fetuses showed chromosomal abnormalities (one 47,XY +18 and two 69,XXX) and were excluded from this study. In 29 cases a subsequent birth weight was available, which helped confirm the diagnosis (birth weight ≤10th percentile). The gestational age at birth ranged from 22 to 39 weeks. The numbers of cases for differing measures vary somewhat because of the small volume of material available, sometimes requiring that some parameters not be analyzed on a given specimen.

Cordocentesis was performed as an outpatient procedure without maternal fasting or sedation or fetal paralysis and was achieved in every case. Fetal and maternal blood samples (300 μl) were collected in heparized syringes, placed into lithium-heparin tubes, and centrifuged for 10 minutes at 2000 rpm. The plasma was collected and stored at − 70° C. Plasma amino acids were measured with an LKB 4151 "alpha plus" amino acid analyzer (LKB Biochrom Ltd., Cambridge). The intraassay coefficient of variation was 6%.

A specific parameter, the fetal concentrating index, was devised to provide a measure of the overall concentrating ability of the fetus. Six normally concentrated amino acids (threonine, valine, methionine, tyrosine, phenylalanine, and lysine) were selected, and the numeric mean of their fetal/maternal ratios was calculated; this constitutes the fetal concentrating index. The choice of these six amino acids, including both essential and nonessential amino acids in which transplacental passage is mediated by differing mechanisms, is somewhat empiric. However, by using the amino acids that are normally the most concentrated and therefore presumably require the most energy for transport, we believe we can develop the system that is most likely to reflect energy changes in placental function.

Approval from the Hospital Ethics Committee was obtained both for the indication of cordocentesis and for the extra blood taken from mothers and fetuses for this study.

Results

Plasma amino acids from 75 normal control fetuses showed very little variation when grouped between 16

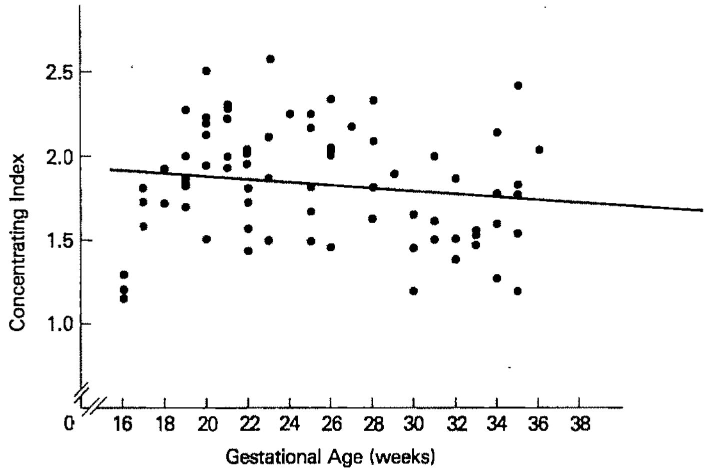

Fig. 1. Amino acid concentrating index as function of gestational age for 75 pregnancies with AGA fetuses. Concentrating index is defined in text. Regression line follows equation $y = -0.0086x + 2.05$. Its correlation coefficient was −0.15.

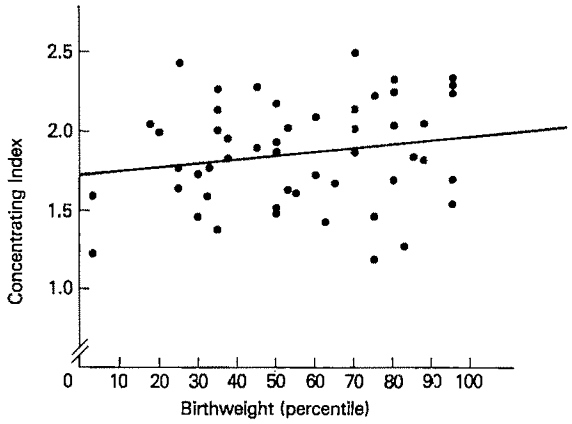

Fig. 2. Amino acid concentrating index as function of birth weight percentile for 51 pregnancies with AGA fetuses. Equation of regression line was $y = 0.0025x + 1.72$. Correlation coefficient was 0.19.

and 36 weeks' gestation (Table I). Two exceptions were asparagine, which was much lower in the second trimester than in the third trimester, and glutamine, whose mean plasma concentration fell with advancing gestational age. Fetal plasma free and total carnitine concentrations did not vary with gestational age (Table I).

Several essential amino acids such as threonine, valine, methionine, and lysine, as well as the presumably nonessential amino acids tyrosine, arginine, and ornithine, exhibited elevated fetal/maternal ratios in all gestational age groups (Table II). These amino acids are apparently concentrated by the fetus. Most nonessential amino acids, as well as free and total carnitine, are not concentrated in the fetal circulation. For most amino acids, as well as free and total carnitine, the fetal/maternal ratio did not vary with gestational age (Table II). The fetal/maternal ratio for valine, threonine, proline, and tyrosine decreased with advancing gestational age, whereas that for arginine increased.

With the fetal concentrating index, as defined in the Methods section, comparisons could be made between the growth-retarded and control fetuses. For normal control pregnancies the fetal concentrating index was 1.83 ± 0.42 (mean ± SD) and did not vary significantly with gestational age (Fig. 1) or birth weight percentile (Fig. 2).

To ascertain the potential contribution of mitochondrial fatty acid oxidation to energy production in developing fetuses, carnitine's concentrating ability was compared with amino acid's concentrating ability. Thus the fetal/maternal ratio of free carnitine was plotted against the fetal concentrating index for normal pregnancies (Fig. 3, *A*). If free carnitine were concentrated in the fetal circulation, a positive correlation would be expected. No such correlation was observed, indicating that the very same fetoplacental units that concentrated several amino acids nevertheless failed to concentrate free carnitine. There was also no correlation between the fetal/maternal ratio for free carnitine and the fetal concentrating index in 24 pregnancies with SGA fetuses (Fig. 3, *B*). Similar results were obtained when total rather than free carnitine ratios were studied in either AGA or SGA fetoplacental units (data not shown).

Pregnancies with AGA and SGA fetuses were

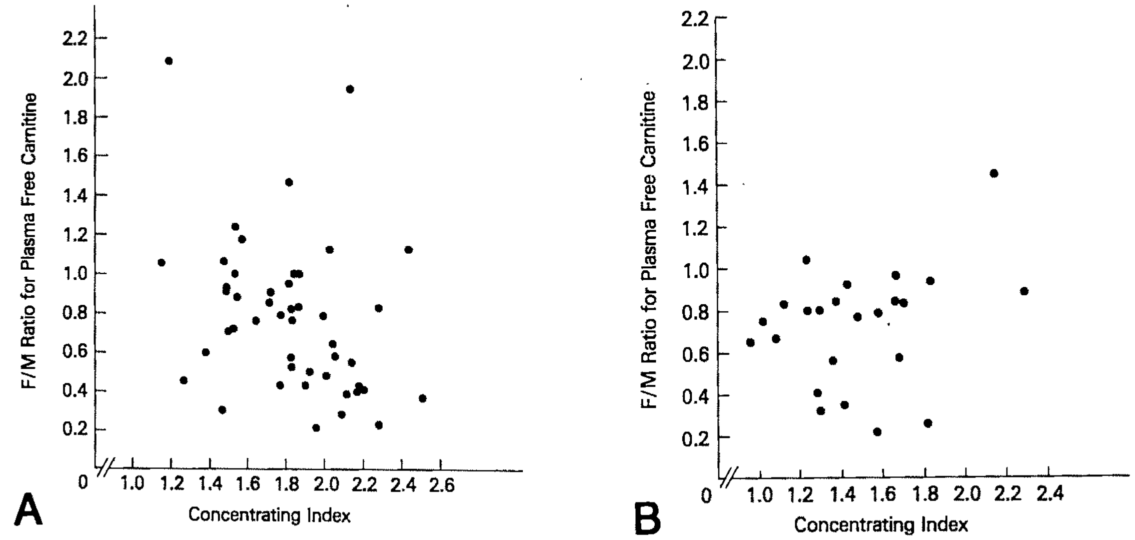

Fig. 3. Fetal/maternal ratio of plasma free carnitine plotted against concentrating index for 47 AGA (**A**) and 24 SGA (**B**) fetomaternal units. No correlation was noted for either group.

Table III. Fetal/maternal ratios of free and total carnitine concentrations and concentrating index for pregnancies with AGA and SGA fetuses

	AGA (mean ± SD)	*SGA (mean ± SD)*	*p**
Free carnitine fetal/maternal ratio	0.78 ± 0.37 (*n* = 45)	0.73 ± 0.27 (*n* = 24)	NS
Total carnitine fetal/maternal ratio	0.94 ± 0.25 (*n* = 19)	0.82 ± 0.23 (*n* = 14)	NS
Concentrating index	1.83 ± 0.42 (*n* = 74)	1.46 ± 0.38 (*n* = 41)	<0.001

NS, Not significant at $p = 0.05$.
*Two-tailed Student's *t* test.

grouped and compared with respect to their fetal/maternal ratios for amino acids and carnitine. Predictably, the ratios for free and total carnitine in pregnancies with SGA fetuses did not differ from normal ones (Table III). In contrast, however, the fetal concentrating index was significantly reduced in the pregnancies with SGA fetuses (1.46 ± 0.38), indicating impaired ability to concentrate certain amino acids in the IUGR fetuses. In fact, seven of 38 pregnancies with SGA fetuses had a fetal concentrating index <1.10, compared with none of 75 normal fetomaternal pairs (χ^2, 18.54; $p < 0.00001$). The fetal concentrating index for 38 pregnancies with SGA fetuses did not vary with gestational age (Fig. 4). A sensitivity of the fetal concentrating index for SGA fetuses was 100% with a specificity of 71%.

Comment

Previous expressions of fetal plasma amino acid concentrations have been in terms of a mean for 26 weeks' gestation and a slope to express variation with gestational age. Grouping by gestational age (Table I) allows ready determination of the normalcy or abnormalcy of an individual fetal amino acid concentration, determined at a particular gestational age, especially when the normal values change with gestational age. The grouping of fetal plasma amino acid values provides more accessible control values for comparison with those of fetuses at risk for aminoacidopathies and also can be used to monitor maternal dietary control in disorders such as phenylketonuria. The values also provide a reference table for comparison with amniotic fluid,[8, 9] cord blood,[8, 10] and plasma from infants,[8, 11] children,[8] and adults.[8, 12]

The fetal circulation does not concentrate carnitine, or β-hydroxy-δ-trimethylaminobutyric acid. This small molecule transports long-chain fatty acids into the mitochondria for oxidation to produce energy.[13] Apparently, the maternal concentration of free carnitine, which is lower than in nonpregnant women,[14-16] is sufficient to serve the fetal needs for fatty acid oxidation throughout pregnancy. In fact, no fetus with IUGR had a markedly decreased plasma carnitine level as a putative cause of growth retardation. The reason for re-

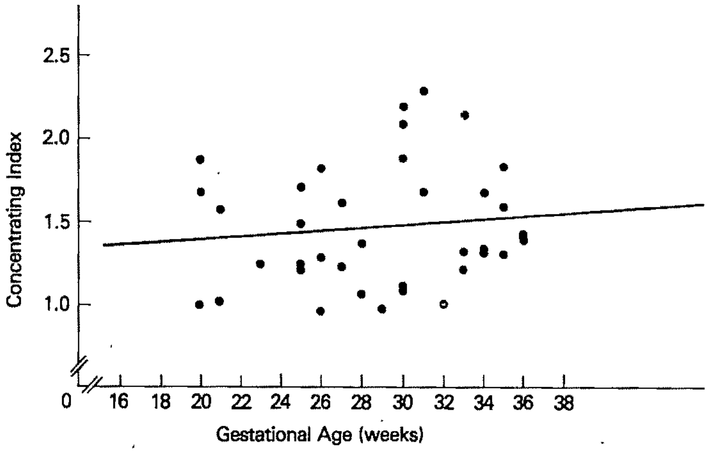

Fig. 4. Amino acid concentrating index as function of gestational age for 38 pregnancies with SGA fetuses. Regression line follows equation $y = 0.0091x + 1.195$. Correlation coefficient was 0.12.

duced maternal plasma carnitine in pregnancy remains enigmatic but may be related to an enhanced volume of distribution without increased hepatic synthesis or intestinal absorption of carnitine.

Our main focus, however, is the introduction of the fetal concentrating index as a more powerful measure of fetal amino acid's concentrating ability. Unlike measurements of individual amino acids that need to be adjusted for gestational age and as a whole do not differ between AGA and SGA fetuses, the fetal concentrating index is very significantly reduced in SGA fetuses compared with normal controls (Table III). Thus in the future the fetal concentrating index might be used as a quantitative gauge of the integrity of the fetoplacental unit when factors causing placental insufficiency are being investigated.

One major advantage of the fetal concentrating index is its gestational age independence; uncertainties about gestational age are rendered irrelevant. Furthermore, since a reduction can be observed even in early gestation in fetuses who develop IUGR, it may be possible to predict the cause of IUGR on the basis of this index. As cordocentesis has become very safe in experienced hands, it may now be ethical to use the fetal concentrating index to predict impending placental compromise, with consideration of early intervention in at-risk pregnancies. Amino acid determinations performed in an experienced laboratory and fetal concentrating index calculations can be offered in attempts to make this parameter part of a practical approach to IUGR diagnosis and potential intervention.

The choice of the six amino acids used in the fetal concentrating index is certainly arbitrary. Conceivably with more experience the choice, number, or relative weighing of components might change. Until a better understanding is obtained, we will continue to collect all parameters. Similarly, further work is needed to correlate these data with other diagnostic parameters such as biophysical and biochemical measurements.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. JACQUES F. ROUX, Jackson, Wyoming. In the past 20 years maternal and fetal plasma amino acid profiles and concentrations have been determined in umbilical cord blood of normal deliveries and cesarean sections.[1, 2] In recent years, with the use of ultrasonography, cord blood is obtained in utero. This procedure is called cordocentesis or percutaneous blood sampling. It has to be noted that Cetin et al.[3] in 1988 and Economides et al.[4] in 1990 used a similar research protocol to determine amino acid concentration. They observed a decrease in amino acid concentration in the cord blood of SGA fetuses. Carnitine concentrations in fetal blood were measured, and no major comments need to be made about this substrate of fatty acid oxidation.

In this article the data have been broken down by gestational ages, including single amino acid concentration and fetal versus maternal level of amino acid. The results are expressed as a fetal/maternal ratio. When the ratio is >1, there is active transport of maternal amino acids to the fetal compartment. I have difficulty accepting some of the statements without a statistical evaluation of the data, especially those on asparagine and glutamine. Each standard deviation is very large and differs for each amino acid. This indicates that each substrate has its own metabolic pathway and that, the concentration differences not being significant among age groups, the data can be pooled. The authors should study one amino acid at a time in well-defined metabolic circumstances. For instance, asparagine and glutamine might be of interest since they show changes that seem to be statistically significant. Glutamine is the precursor of acetoacetate, a citric acid substrate, necessary to the Krebs cycle to provide energy to the fetal cells in the form of adenosine 5'-triphosphate. Asparagine, like alanine, goes to pyruvate and then to glucose and is a precursor of glycoprotein, a component of fetal membranes. Therefore the measurement of these two amino acids would provide interesting information on the fetal-placental unit, and the results might be more meaningful to the clinician than an indiscriminate screen of amino acids.

The present results are compared with maternal blood obtained in the upper extremity. The maternal concentration of amino acids could be misleading since muscles release various amounts of this substrate in response to food intake, amount of time after ingestion, and exercise. Furthermore, the circadian rhythm for amino acids is characterized by peak level, between 12 and 8 o'clock in the evening and between 4 and 8 o'clock in the morning.[5] These observations give little support to the use of brachial venous blood without a well-defined time of testing. Intervillous blood would be the best standard of comparison.

The computation of the fetal/maternal ratio indicates an active amino acid transport system. The large variability of the standard deviation of this ratio makes it difficult to interpret the data, except that the fetal/maternal ratio of valine, threonine, proline, and tyrosine decreases with advancing age, but there is no statistical study of the data. The results could be due to an increase in maternal amino acid concentration with gestational age. This would increase the denominator of the fetal/maternal fraction and produce a decreased ratio.

The authors stated that they had difficulty in developing a meaningful clinical test for amino acids. They decided to average the fetal/maternal ratio of six amino acids showing a high transport rate. They called it the fetal concentrating index. The idea was an excellent one, if one wanted to focus on transport and as long as one accepted that each amino acid has its own metabolic pathway, as is the case. The authors show that the fetal concentrating index for the SGA fetus is lower than that of the control. They then conclude that the SGA fetus has a deficiency in active placental transport of the six amino acids because of placental asphyxia. Two strong objections can be made to these statements. First, the difference between the fetal concentrating index means is 20%. Clinically this is meaningless because the statistical significance is mainly due to the number of determinations. Therefore, from the clinical point of view, it may be true that in a large population of SGA fetuses the fetal concentrating index will be low; however, on a case-to-case basis it will be difficult to consistently demonstrate a significant variation among samples. A normal physiologic variation can be as large as 15% to 20%. Second, in this clinical sampling one does not know how placental asphyxia was evaluated. In each case fetal acid-base status should have been determined and the data correlated with the fetal/maternal ratio. In this regard determinations on intervillous blood samples would be rewarding. Without this information the authors' conclusions are open to question because they are not verified empirically.

From a theoretical point of view the metabolism of amino acids supports the observations of the authors. In adults amino acids are transported actively through membranes by glutamyl transpeptidase.[6] This is an energy-dependent mechanism. Therefore a decreased fetal/maternal ratio could be explained by a lack of adenosine 5′-triphosphate generation, as a secondary result of a low Po_2.

In man, muscle releases alanine, which is transported to the liver, which converts it to glucose.[7, 8] Therefore, if the amino acid–glucose cycle is active in the placenta, a decrease in placental blood amino acid concentrations could reduce fetal amino acid availability. It is known that human placental tissue actively transports amino acids in vitro and in vivo. It uses them for its own metabolic needs and releases them in the fetal circulation. The mechanism of release was studied by Schneider et al.[9] He showed that placental tissue released only 26% to 47% of its amino acid content in an amino acid–free medium. It is then possible that the classic amino acid–glucose cycle observed in muscle and liver is different in the placenta. For instance, the decrease in amino acid level in the fetus could be due to a change in turnover of substrates in the fetal blood, the liver extracting a greater amount in the SGA fetus than in the normal fetus. Another possibility is that the SGA fetus releases less amino acid into the circulation from the placenta, muscle, and liver. This is independent of an abnormal amino acid transport. I strongly suggest to the authors that they expand their investigation to placental tissue and fetal-maternal blood obtained under ultrasonographic guidance. Blood glucose, pyruvate, amino acids, glucagon, insulin, and α-ketoglutarate concentration should be determined under controlled conditions to avoid biologic variations. These data should then be correlated with the maternal-fetal Po_2 and the acid-base balance in each case. A placental membrane preparation should be investigated for the presence of glutamyl transpeptidase in the SGA and normal fetus.

In the meantime, the present data interpretation is doubtful and the clinical applications are very limited. This study helps us to formalize new work hypotheses and to have a set-of-data basis. It opens new vistas on fetal biochemistry, a field that has been somewhat neglected in the last 10 to 15 years.

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DR. RALPH K. TAMURA, Chicago, Illinois. With this study Bernardini et al. are extending and elaborating on significant prior work done in this area. Having previously published data on fetal plasma amino acid profiles in SGA and AGA fetuses, they now report on the usefulness of adding carnitine levels, and they have developed a coordinated parameter that they refer to as the fetal concentrating index to quantitatively describe the ability of the placenta to extract circulating amino acids. It appears to me that the patient data used for this study are identical to those previously reported by the authors,[1, 2] except of course for carnitine levels and for the fetal concentrating index. The following are queries that hopefully may clarify and strengthen this paper:

1. For the control pregnancies studied, could the fetal disease in any way affect the amino acid levels of the umbilical venous blood?

2. Why were fetuses with encephaloceles, hydrops, or chromosomal defects excluded? What are fetal concentrating indexes in these fetuses?

3. Regarding the number of control pregnancies, 75 minus 20 (16 terminations, 4 lost to follow-up) equals 55, not 57.

4. Regarding the cordocentesis procedure, how many attempts were performed per patient to obtain an adequate sample?

5. Why are there 80 control fetuses in the Results section but 75 in the Methods section?

6. Should not mention of the fetal concentrating index be placed in the Methods section?

7. Have these investigators analyzed the effect of using different individual amino acids for different gestational ages? That is, might not a different set of amino acids improve the predictive value of the fetal concentrating index in different trimesters?

8. Why were ornithine and taurine (high fetal concentrating index values) not used as part of the fetal concentrating index?

9. In the Methods section, 31 SGA fetuses were studied. Why were there 38 SGA pregnancies in the Results section?

10. May we asume that, on the basis of the data provided, the predictive value of the fetal concentrating index for SGA fetuses is 7 of 38 (18%) with a fetal concentrating index cutoff value of 1.10? What are sensitivity and specificity values?

This is an extremely interesting study in which basic and clinical research appear to have coalesced hopefully to provide some meaningful insights into the understanding of IUGR and SGA fetuses. To be used as part of the clinical armamentarium, however, correlations will need to be made between the fetal concentrating index and other antenatal diagnostic tests (i.e., biophysical profile, nonstress testing, and Doppler velocimetry, all of which are noninvasive).

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DR. KENNETH J. MOISE, JR., Houston, Texas. I was wondering if you tried to correlate the ultrasonographic weights with the fetal concentrating index, both in the normal population and in the IUGR population? You appeared to be talking about babies after they were born as being ranked in the 5th percentile. Was there any correlation between the fetal concentrating index and the actual ultrasonographic weight at the time of the fetal blood sampling and the same question for IUGR?

DR. RICHARD P. PERKINS, Omaha, Nebraska. I am trying to decide what the importance of this is. First, it depends on whether you are of the school of thought that to detect it very early is a good thing. If one pursues that logic to its conclusion, one would say that cordocentesis is a procedure that is essentially without risk in the most experienced hands, that the objective therefore is to do it at the earliest point possible, and that we should have all of our cordocenteses done by only one person.

DR. CHIN-CHU LIN, Chicago, Illinois. I enjoyed the study very much. Briefly, in fetal growth retardation one of the mechanisms is thought to be a placental malfunction of the transport mechanism in the placenta for essential nutrients across the membranes.

My question is, have you made any correlation with the concentration of fetal-to-maternal rate gradient with respect to simple measures, such as placental morphologic type, either grossly or histologically?

DR. CHESTER B. MARTIN, Madison, Wisconsin. To follow up on the last question, were there any differences in an outcome variable between those SGA fetuses with a fetal concentrating index <1.1 and >1.1? And how many inadvertent or unplanned interventions were carried out as a consequence of the cordocentesis?

DR. PETER F. FEHR, St. Paul, Minnesota. One of the problems experienced with patients who had intravascular transfusions is fetal-maternal bleeding. I'm wondering if the authors measured these and whether they discovered, as our areas discovered, that synthesis is occurring in these patients at a higher rate than in the normal population. How would they justify this in terms of maternal informed consent before they do a cordocentesis in these patients?

DR. WILLIAM J. OTT, St. Louis, Missouri. I have a number of questions, but I will limit them to two. In your Methods section, how did you differentiate between the SGA infant who suffers from IUGR and those infants that are small for gestational age but are so because they are constitutionally small but otherwise totally normal? Second, how did you differentiate between the AGA fetuses whose birth weights may be above the 5th percentile but still have not met their inherent growth potential?

The second question deals with the clinical relevance of the study. Although cordocentesis may provide important biochemical data, it is an extremely operator-intense procedure. Our institution has been using the technique for approximately 2½ to 3 years, and we find that it significantly raises catecholamine levels in both the mother and the physician. I wonder whether the information obtained from cordocentesis provides any additional information over that obtained from noninvasive procedures, such as fetal heart rate monitoring or Doppler blood flow studies?

DR. EVANS (Closing). I thank all of the discussants and questioners for their thoughtful comnents.

Probably the most important concept I would like to reemphasize is that this is a pilot study. We felt it was necessary to do these determinations on patients who were having cordocentesis for other indications and use extra blood to see if we could define a parameter that would help us. I think we have to some degree.

I agree with Dr. Roux that there are a number of different possible explanations for the specific differences seen. It is also not clear that these six amino acids that we have chosen through our first pass will end up being the ultimate parameters that are used, if any, as this data set continues to go on. The major thing we tried to accomplish with this article was to show optimism that we might have a useful parameter and to make it ethically appropriate to attempt to study this prospectively.

None of the management of patients was affected by this, because these tests were conducted long after the pregnancies were concluded. As a matter of fact, they were run on different sides of the Atlantic from where the patients actually were, so it would be hard to have an exact intervention on the basis of the results.

In terms of what Dr. Roux said about where we draw the blood, it might be better scientifically to get the blood out of the femoral artery. I think politically it would be much less acceptable and much less likely to be allowed to be used.

Dr. Tamura, as far as numbers are concerned, obviously I'm not as good at the abacus as you are. There are a couple of differences mainly because we did not have all the data for some of the patients because there was a variable amount of blood available for running the 30 or so different parameters. So for some patients it turns out we had enough blood to run for the parameters that ultimately became the fetal concentrating index but not enough for other parameters.

A 1.1 cutoff was arbitrarily defined but did pick up, as Dr. Tamura said, in seven out of 38 cases. Whether that will be the ultimate cutoff will depend on how many more numbers are necessary to have an index.

As for Dr. Moise's question, the patients were categorized at the time of the cordocentesis as to the ultrasonographic findings, and the results were confirmed post partum; only patients who fell into the same category twice were used.

Dr. Lin asked about discordant twins. We have a couple of samples from those in the freezer, but we haven't run them yet.

Dr. Ott also asked about distinguishing between IUGR and SGA fetuses; it is true that we can't distinguish the exact cause in all those patients, but again we're trying to work on it.

There were no complications from cordocentesis in this particular series. Certainly, depending on the published series, when complications have been as high as 5% to 7% or as low as 0.5% to 1%, I think that as experience becomes greater the complication rates will go down to a point where a number of people will be able to do this procedure with safety.

I think that the issue of fetal-maternal bleeding is certainly one issue that needs to be addressed and one that needs to be looked at. We did not look at it nor were there any particular questions.

Another question was asked regarding control. The control patients were mostly patients who were being tested for diseases such as hemoglobinopathies but were found not to be affected. Anatomic defects such as limb reduction defects were felt not to be likely to affect the plasma amino acids.

Patients who had more serious internal disorders, such as hydrops, encephalocele, etc., for whom we did have a serious concern but certainly no data that there might be some impact on the circulating amino acids, were not included in this study for precisely those reasons.

As the numbers build up over time, we can probably begin to subcategorize patients by the different areas, but at this point the quickest thing to do was merely to categorize patients as easily as possible.

Drug abuse and other risk factors for physical abuse in pregnancy among white non-Hispanic, black, and Hispanic women

Abbey B. Berenson, MD,[a] Norma J. Stiglich, MD,[a] Gregg S. Wilkinson, PhD,[b] and Garland D. Anderson, MD[a]

Galveston, Texas

We investigated prevalence and risk factors associated with physical abuse among a tricultural population of 501 pregnant women who attended a public prenatal clinic. Twenty percent (98/501) reported being physically abused; 29% (28/98) stated that abuse occurred during pregnancy. More white non-Hispanic women reported previous abuse than did black or Hispanic women. Battered women more frequently were divorced or separated, were of greater parity, smoked, used alcohol, or admitted to illicit drug use than did women who were not battered. An increased risk of previous physical abuse was observed among white non-Hispanic and black women who used alcohol (odds ratios = 3.0 and 6.0) or drugs (odds ratios = 2.1 and 3.7) but not among Hispanic women. Odds ratios of 4.7 for cocaine use among white non-Hispanic women, 4.7 for marijuana use among black women, and 5.8 for tobacco use among Hispanic women were observed. This is the first study to report the effects of race on the association between physical abuse of pregnant women and sustance use. (AM J OBSTET GYNECOL 1991;164:1491-9.)

Key words: Battering, physical abuse, spouse abuse, drug use, alcohol use, tobacco use

Physical abuse has been reported to occur at least once in 28% of all United States marriages with 3.8% of all couples admitting to more violent acts than pushing, slapping, kicking, or throwing objects.[1] Most reports on abuse are derived from women in shelters, crisis centers, or emergency rooms. Few studies have addressed the prevalence of violence in a pregnant population, even though pregnancy has been suggested as a risk factor with violence either beginning or increasing during this period. Hillard[2] noted a history of physical abuse in 81 of 742 obstetric patients. Helton et al.[3] interviewed 290 women in both public and private prenatal clinics and noted a 23% prevalence with 8% reporting abuse in the current pregnancy.

Risk factors other than pregnancy reported to be associated with abuse have included alcohol or drug dependence in the male partner, violence in the family of origin of either partner, low socioeconomic status, and unemployment. Few have investigated the victim's use of illegal drugs. Kantor and Straus[4] examined marijuana use in 2033 women and noted that severely assaulted women had a usage rate six times higher in the year preceding their emergency room visit than did nonvictims but concluded that drug use among wives was not significant as a risk factor for physical abuse. In a smaller study of 49 battered women, Bergman et al.[5] noted that 16% had a history of intravenous use of psychostimulants or heroin, as compared with 2% of nonvictims, but did not further classify the drugs by type. Recently, Amaro et al.[6] reported that victims of abuse during pregnancy were more likely to use marijuana, cocaine, and alcohol than were nonvictims. However, the effects of race on the relationship between drug or alcohol use and violence during pregnancy were controlled for rather than investigated in their analysis.

The purpose of this study was to measure the prevalence of battering in a large tricultural, public, prenatal clinic population and to determine risk factors within each race–ethnic group associated with physical abuse, including the victim's use of alcohol and illegal drugs. The type of drugs used also was investigated.

Material and methods

All patients ≥18 years old who attended the university's low-risk obstetric clinic between May 8, 1989, and Feb. 5, 1990, were interviewed by their clinician for a history of physical abuse at their second visit. Eighty percent were interviewed by a physician's assistant or nurse practitioner, 15% by a resident, and 5% by a medical student. A modified version of Helton's ques-

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tionnaire in Spanish and English was used, with permission, to ensure uniformity in questioning.[3] Thirty-one patients who presented during this period were not surveyed because of unavailability of a translator (one patient), refusal (one patient), or an oversight by their clinician (29 patients). After confidentiality was emphasized, 501 adults were asked privately, "Has anyone hit, slapped, kicked, or otherwise physically hurt you?" Positive responders were questioned on their relationship to the attacker, the last date of occurrence, and the body site injured at that time. Women were asked whether medical care had been sought and if battering had occurred or increased during the current pregnancy. Practical information on legal rights and community resources was disseminated to all patients. An on-site social worker counseled those with a history of physical abuse.

Women were also queried as to their use of tobacco and alcohol since becoming pregnant and whether they had used illegal drugs at any time during the last 12 months. Sociodemographic data were obtained by reviewing each patient's hospital records with institutional review board approval. Thirty-three percent of charts were reviewed a second time to assure accuracy of data entry.

The χ^2 test of independence was used to determine whether statistically significant differences were present between physical abuse and demographic characteristics. The Student *t* test was used to determine whether significant differences existed between continuous variables such as age and the presence of abuse.

After measuring the prevalence of physical abuse, we calculated the odds ratio of physical abuse relative to nonabuse by race, language spoken, marital status, employment, and use of substances including tobacco, alcohol, and illicit drugs. The prevalence odds ratio estimates the risk ratio and provides a measure of the strength of the association between the risk factors being examined and the frequency of physical abuse.[7] We also calculated 95% confidence limits on the basis of a maximum likelihood procedure that was first described by Cornfield[8] and later refined by Gart.[9]

Stratified analyses were conducted to determine the effect of race–ethnic background on the risk of physical abuse by use of tobacco, alcohol, or drugs. Asian patients were not included in the analysis by race because an inadequate number were available for comparison. Both unadjusted and age-adjusted odds ratios were calculated. A stratified maximum likelihood procedure was used to adjust for age differences.[10] The likelihood ratio test for heterogeneity was applied to the stratum-specific results to ensure the applicability of the maximum likelihood approach to age adjustment.[11] Ninety-five percent confidence limits around the adjusted odds ratios are reported.

Results

Five hundred one patients were interviewed. The population consisted of 47.5% (238/501) white non-Hispanic, 28.1% (141/501) black, 24.0% (120/501) Hispanic, and 0.4% (2/501) Asian women. Patients ranged in age from 18 to 43 with a mean (±SD) of 23.5 ± 4.5 years. Ninety-two percent (459/501) were surveyed in English and 8% (42/501) in Spanish. The 31 patients not surveyed did not differ significantly from the study population by race, age, gravidity, parity, or gestational age.

Twenty percent (98/501) of the women questioned reported having been battered (95% confidence limits = 16.1, 23.0). Twenty-nine percent (28/98) of the victims stated that abuse had occurred in the current pregnancy with six noting an increase in violence during pregnancy. Nineteen of the 98 battered women had sought medical attention.

The face was the most frequent site injured and extremities the second most common. Seven of the fourteen women who reported being struck in the abdomen were pregnant when hit. Ninety-two percent (88/96) of the women reported a single perpetrator. This was her boyfriend or spouse in 83% (73/88) of the cases. Eleven patients identified a parent as the perpetrator, two had been struck by a brother, one by a brother-in-law, and one by a non–family member. Eight patients identified more than one abuser, two of whom had been hit by a parent and a boyfriend, four by two parents or guardians, one by her exspouse and current boyfriend, and one by her boyfriend and her sister.

In patients who admitted being abused, mean gravidity and mean parity were significantly higher than in those who had not been abused ($p \leq 0.001$, $p \leq 0.001$; Table I). Battered women had more living children than nonabused women ($p \leq 0.001$). No significant differences were noted in maternal age or gestational age between women with and without a history of battering.

The prevalence of reported physical abuse was 3.5 times higher among white non-Hispanic women than it was among Hispanic women (95% confidence limits = 1.8, 6.8) and 1.6 times higher among white non-Hispanic than among black women (95% confidence limits = 1.0, 2.7). Similarly, women with a history of physical abuse were more likely to converse in English than in Spanish ($p = 0.006$).

Battered white non-Hispanic women were twice as likely and battered black women were 15 times as likely as nonbattered women to be divorced or separated when compared with women who reported a negative history of physical abuse (Table II). Married Hispanic women, on the other hand, were more likely than unmarried women to report physical abuse.

When employment status was considered, black women who had been battered were twice as likely to be unemployed than were black women who had not been battered (Table III). An association was observed between battering and status as a student among Hispanic women; however, this finding was based on only one observed case.

Table IV shows associations between physical abuse and substance use by race–ethnic group for women who reported having been battered compared with those who did not report having been battered. Abused white non-Hispanic women had 2.1 times the risk for using drugs when compared with women who had not been abused. Black women who had been battered, however, were 3.7 times as likely to have used drugs than were black women who had not been battered. None of the Hispanic patients who had been physically abused admitted to using drugs.

Age-adjusted relative risk estimates for alcohol use were elevated among white non-Hispanics (odds ratio = 3.0) and blacks (odds ratio = 6.0) but not among Hispanics (odds ratio = 1.0). The relative risk for tobacco use was elevated among all three race–ethnic groups with abused Hispanic women having 5.8 times the risk of non-abused Hispanic women followed by black and white non-Hispanic women, respectively. We also calculated crude odds ratios and pooled odds ratios that we adjusted (using maximum likelihood estimation) for the effect of race on the relationship between physical abuse and substance use. Because the race–ethnic group specific odds ratios are so different, the crude estimates are probably of less interest in this setting.

Table V shows the relationships between the type of substance used among abused and nonabused women by race–ethnic group. The relative risk of cocaine use was 4.7 for white non-Hispanic women who had been physically abused when compared with those who had not been abused. Abused and nonabused black women did not differ in their use of cocaine. The relative risk for marijuana use among abused black women, however, was 4.7 whereas the risk for marijuana use among white non-Hispanic women was 2.7. No black woman who had been battered reported using both marijuana and cocaine, but the relative risk for using both of these drugs was 2.4 among battered white non-Hispanic women.

Comment

Pregnancy has been cited as a risk factor for the initiation or escalation of physical abuse. Reasons hypothesized for abuse during pregnancy include violence directed toward the fetus, sexual frustration, stress of family transition, biochemical changes, and defenselessness of the pregnant woman.[12] More than one fourth of our patients with a history of abuse reported violence in the current pregnancy with an escalation in battering noted by six women. This study, however, most likely underestimates the number of women who are abused in pregnancy because interviews were conducted at the second prenatal visit. Since the violence cycle includes a remorseful phase with promises by the man that abuse will not be repeated, women interviewed during this phase may erroneously report that abuse has ceased. Five women, who had been struck within the last year, stated that they were not currently being abused and had not been hit in the current pregnancy, although they were still involved with abusive partners. If the interview had been conducted later in gestation, a higher prevalence of abuse during pregnancy may have been detected. However, a later survey would not have detected battered women who aborted or had a preterm delivery. The most effective screening program would include more than one interview in pregnancy.

Table I. Demographic characteristics of battered pregnant women compared with pregnant women who were not battered

	Battered	*Not battered*	*p Value*
Age (yr)	24.1 ± 4.8	23.3 ± 4.4	NS
Gravidity	3.1 ± 1.2	2.5 ± 1.6	≤0.001
Parity	1.5 ± 1.1	1.0 ± 1.2	≤0.001
Living children	1.5 ± 1.1	1.0 ± 1.2	≤0.001
Gestational age at interview (wk)	22.0 ± 9.0	22.5 ± 9.6	NS

Data are presented as mean ± SD.

We found that white non-Hispanic women were more likely to report previous battering than were blacks or Hispanics. In contrast, Helton et al.[3] found no racial differences in reported abuse. In our study Hispanic women also admitted using alcohol and drugs less frequently than did white non-Hispanic and black women. This analysis was dependent on self-reporting; therefore bias could be present if Hispanics were less likely to discuss these events openly with their clinicians. Recall bias of an event as traumatic as physical abuse is unlikely to be dependent on racial or ethnic status. Because the study population comprised pregnant women only, pregnancy itself would not contribute to recall bias since both abused and nonabused women were pregnant at the time of interview.

Although the face was the most common site hit both before and during pregnancy, the abdomen was hit almost twice as frequently in pregnant women as compared with those who were not pregnant when battered (27% vs 14%). This may represent prenatal child abuse and may result in an increased risk of an adverse outcome. Morey et al.[13] described an infant with an intraventricular hemorrhage and extensive bruises born to

Table II. Relative risks for different marital status categories by race–ethnic group for battered pregnant women compared with pregnant women who were not battered

Race	*Marital status*	*Battered*	*Not battered*	*Odds ratio*	*95% Confidence limits**
White, non-Hispanic	Divorced-separated	18	24	2.0	1.0, 4.1
	Single	10	62	0.4	0.2, 0.9
	Unmarried*	28	86	0.9	0.5, 1.5
	Married	34	90	1.0	
Hispanic	Divorced-separated	1	8	1.1	0.2, 8.0
	Single	2	29	0.6	0.1, 2.7
	Unmarried*	3	38	0.7	0.2, 2.6
	Married	8	71	1.0	
Black	Divorced-separated	7	11	15.3	2.1, 104.4
	Single	17	81	5.0	0.8, 30.8
	Unmarried*	24	92	6.3	1.0, 37.9
	Married	1	24	1.0	
All races	Divorced-separated	26	43	2.6	1.5, 4.7
	Single	29	172	0.7	0.4, 1.2
	Unmarried†	55	216	1.1	0.7, 1.7
	Married	43	185	1.0	

*Cornfield's maximum likelihood estimate.

†All unmarried combined.

Table III. Relative risks for employment status by race–ethnic group for battered pregnant women compared with pregnant women who were not battered

Race	*Employment status*	*Battered*	*Not battered*	*Odds ratio*	*95% Confidence limits**
White, non-Hispanic	Student	3	17	0.6	0.2, 2.0
	Unemployed	36	95	1.2	0.6, 2.3
	Employed	18	56	1.0	
Hispanic	Student	1	5	2.7	0.3, 26.2
	Unemployed	7	73	1.3	0.3, 5.8
	Employed	2	27	1.0	
Black	Student	2	13	1.4	0.3, 7.6
	Unemployed	15	57	2.4	0.8, 7.5
	Employed	4	37	1.0	
All races	Student	6	35	0.9	0.3, 2.2
	Unemployed	58	225	1.3	0.8, 2.2
	Employed	24	120	1.0	

*Cornfield's maximum likelihood estimate.

a mother who was struck in the abdomen 1 week before delivery. Bullock and McFarlane[14] noted a significant correlation between battering and low birth weight in a survey of 589 adults. Further study is required to determine if physical abuse in pregnancy is associated with an adverse perinatal outcome.

Risk factors previously reported to be associated with physical abuse, in general, include being divorced or separated, being unemployed, and alcohol or drug use by either partner. Hillard[2] reported that abused pregnant women are more likely to be divorced or separated than are women who do not have a history of abuse. Appleton[15] reported that women battered multiple times were three times more likely to have been divorced than were controls. We observed that the association between physical abuse and marital status may be modified by race. Battered white non-Hispanic and black women were more often divorced or separated when compared with nonabused women of the same race. In fact, all categories of unmarried black women were at greater risk for a history of physical abuse than were married black women. Abused Hispanic women, in contrast, were more likely to be married than were those with a negative history. These findings suggest that a victim's response to an abusive situation is likely to be influenced by her culture, and in particular, Hispanic women may be more reluctant to leave an abusive partner than are white non-Hispanic or black women.

The protective role of employment outside the home is questionable. Appleton[15] did not note a difference in employment outside the home between abused and nonabused women. Gelles[16] observed that employment

Table IV. Relative risks for substance use by race–ethnic group for battered pregnant women compared with pregnant women who were not battered

Race	*Substance used*	*Battered*	*Not battered*	*Odds ratio*	*Adj odds ratio**	*95% Confidence limits†*
White, non-Hispanic	Drugs	11	18	1.9	2.1	0.9, 4.3
	Alcohol	20	25	3.1	3.0	1.5, 5.9
	Tobacco	32	81	1.3	1.3	0.7, 2.4
Hispanic	Drugs	0	2	0.0	0.0	
	Alcohol	1	10	1.1	1.0	0.2, 7.0
	Tobacco	4	10	6.5	5.8	1.5, 22.1
Black	Drugs	6	8	4.4	3.7	1.2, 12.0
	Alcohol	11	12	6.6	6.0	2.3, 16.3
	Tobacco	8	20	2.2	1.8	0.6, 4.8
All races	Drugs	17	28	2.9		1.2, 4.6
	Alcohol	32	47	3.8		2.3, 6.4
	Tobacco	44	111	2.2		1.1, 2.9

*Adjusted for age by maximum likelihood estimation.
†Cornfield's maximum likelihood estimate.

Table V. Relative risks for type of drugs used by race–ethnic group for battered pregnant women compared with pregnant women who were not battered

Race	*Type of drugs*	*Battered*	*Not battered*	*Odds ratio*	*95% Confidence limits**
White, non-Hispanic	Cocaine	6	4	4.7	1.4, 16.3
	Marijuana	9	14	2.0	0.8, 4.9
	Both	3	4	2.4	0.6, 9.8
Black	Cocaine	1	6	1.0	0.2, 6.7
	Marijuana	4	5	4.7	1.2, 17.8
	Both	0	3	0.0	
Both races	Cocaine	7	10	2.7	1.1, 17.8
	Marijuana	13	19	2.7	1.3, 5.6
	Both	3	7	1.7	0.4, 5.9

*Cornfield's maximum likelihood estimate.

could determine whether a woman sought outside assistance in an abusive situation and noted that wives who do not seek intervention are more likely to be unemployed. We observed that the association between an abuse history and unemployment varied with race with an increased risk of unemployment among battered black women but not white non-Hispanic and Hispanic women.

Our findings of an increased risk of abuse associated with the use of alcohol is consistent with findings reported by other investigators. Bergman et al.[17] reported that 51% of abused women seen in an emergency room and 88% of their assailants were heavy consumers of alcohol. Kantor and Straus[4] noted that alcohol use by either partner was a significant risk factor for abuse, and Eberle[18] found a strong correlation between alcohol use by the victim and physical abuse. Both Coleman et al.[19] and Telch and Lindquist[20] reported significantly more drinking problems in both the husband and wife of violent couples as compared with nonviolent couples. Hillard[2] detected that victims were more likely to report alcohol use than were nonbattered women. Our study of a pregnant population confirms that victims more frequently report alcohol use than do nonvictims.

The role of illicit drug use by the battered female has not been well documented. Coleman et al.[19] noted frequent legal drug use in battered wives. In a study of marijuana use in battered women, Kantor and Straus[4] noted a sixfold increase among victims of severe violence compared with nonvictims. Amaro et al.[6] observed that women hit during pregnancy were 2.7 times more likely to be heavy users of illicit drugs than were nonvictims. Appleton,[15] however, detected illicit drug use other than marijuana in only one subject out of 30 acutely battered women.

This is the first study of which we are aware that has detected differences in the relationship between race, substance abuse, and a history of battering among pregnant women from the three races–ethnic groups: white non-Hispanic, black, and Hispanic. We observed that pregnant women who had been battered reported using illegal drugs more often than women who did not report a history of physical abuse (adjusted odds ratio = 2.9, 95% confidence limits = 1.2, 4.6) and we further noted an increased risk of cocaine, as well as marijuana use, when analyzed by race. The risk of cocaine use was highest among white non-Hispanics as compared with blacks and Hispanics, while marijuana was the most common drug used by battered black women. In addition, white non-Hispanic women more frequently reported using both substances than did blacks or Hispanics.

Limited data are available regarding whether drug use represents self-medication by a depressed, battered woman or whether the environment of a substance abuser later leads to physical abuse. Dembo et al.[21] observed that battered children have lower self-esteem, less confidence, and more mistrust of others and theorized that victims use drugs as an escape from pain. One may speculate that victims of abuse attempt to cope with their situations through the use of drugs, alcohol, or tobacco and may use illegal substances as mood elevators or tranquilizers. Alternatively, the environment of a substance abuser, with its potential for violence, may increase the possibility that battering will be experienced. Drug addiction may further hinder the victim by increasing the difficulty of leaving an abusive partner, especially if he supplies the drug or provides the finances for its purchase. Further study in this area with confirmatory urine testing for drugs is warranted.

In conclusion, a prevalence of 20% for previous physical abuse was reported in a pregnant population with almost one third of the battered subjects reporting abuse during the current pregnancy. Differences between battered and nonbattered pregnant women included race, marital status, employment status, parity, and use of tobacco, alcohol, and drugs with battered women of each race demonstrating a different profile.

Clinicians who provide prenatal care should be aware of the risk factors that place pregnant women at an increased risk for a history of physical abuse. Knowledge of risk factors particular to the patients' race or ethnic group may help identify those who are at risk of physical abuse. However, no stereotype of a battered pregnant woman is available. Therefore all pregnant women should be screened by direct questioning to allow counseling, referral, or even legal action to be invoked before serious injury to the mother or the fetus is experienced.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Richard P. Perkins, Omaha, Nebraska. The authors have presented the results of a substantial interview survey of pregnant women of varied ethnic backgrounds concerning the prevalence of physical abuse before and during pregnancy. The data are well collected and analyzed. They point out that traditional risk factors such as alcohol use in the male, low socioeconomic status, and unemployment were well represented in the study population. These findings are not news. What is stressed is the finding of an association with drug use among the victims.

In this population all patients were at least 18 years old and were "low-risk" obstetric clients (however one can define that in a clinic population). The ethnic groups were of sufficient numbers for meaningful analysis. There was a 20% history of abuse of some sort in the past, and almost 20% of these sought medical attention (or protection) for these incidents. In some cases the abusive behavior increased during pregnancy. In most cases abuse was by one person, usually the mate.

Anglo women reported a greater frequency than black or Hispanic women. English-speaking Hispanics, presumably reflecting Anglo acculturation, reported a higher incidence than others in that ethnic subset. Battering was seen much more often among black women who were unemployed than among black workers.

Drug use, including alcohol, tobacco, and marijuana, was usually increased in Anglos and blacks who reported abuse. Hispanic smokers showed a greatly increased risk. More battered Anglo and black women were divorced than married. Unwed and/or unemployed blacks were disadvantaged in partner treatment, yet the reverse was true for unwed Hispanics. It is very possible that Hispanic women are far less likely to admit to this treatment than others, because in my experience with Hispanic clients there was a lot of this behavior.

The findings are compatible with results of prior studies cited by the authors, yet some fundamental questions remain unanswered. *The single most important of these is, which comes first, the drug use or the battering?* Is the battering reflective of a general pattern of self-abuse, of which drug use is yet another example? Or are these women using drugs to escape the horrors of their lives? Or are they just in a consistent pattern of making the wrong choices for themselves in all areas of their lives? Does drug use make one more prone to battering through the effects of the drug on behavior? If the latter were so, one should not see it in marijuana smokers, I should think, as they are often mellow to a fault. Cocaine abuse, on the other hand, frequently is associated with aggressive behavioral traits.

Alcohol use was especially prevalent among partners of alcohol-abusing women. This is representative of the axiomatic state of life among drug users: It is as likely that only one of a family uses drugs as it would be to have only one cockroach. Do drunks really like each other, as we would be asked to believe sometimes? Most would say no and that they remind them too much of themselves.

The data raise other questions. Do unemployed women experience abuse simply because they are more available to their equally unemployed and frustrated partners?

Of interest, the abused women in this study were of the same average age as controls but had more children. Do children raise the level of tension in a stressed home environment? Undoubtedly they do.

Finally, it should be noted that the abusive partner may be using the only effective decompression mechanism of which he (or sometimes she) is capable. Tensions build and resources for alleviating them through reason, discussion, diversion, or other intellectual avenues fail. Some humans lose what limited articulateness they normally possess during such emotional states. Perhaps we can approach this problem in the future of man through a concerted effort toward teaching our children the values of resolving conflicts peacefully and through discussion instead of by a variety of physical exercises and brandishing of arms. Strength of will must be as attractive as strength of body, especially when the potential for equality of the sexes is at issue. Of course, eminent success in this venture would leave us without any soldiers. Opinions as to the significance of this surely would vary.

I reiterate my greatest curiosity. Of drugs and battering, which is the initial abnormal event?

Dr. Mary Jean Cannon, Chicago, Illinois. Dr. Berenson and her co-workers have undertaken a study to determine the prevalence of physical abuse in a multiracial pregnant population, specifically to identify risk factors for this behavior within racial groups. Their purpose was valid for a number of reasons: First, there is a paucity of information, especially in the last decade, regarding physical abuse of pregnant women in general. Second, physical abuse in pregnancy is of concern to all obstetricians, particularly in its less recognizable forms. The identification of risk factors to alert physicians to this problem could prevent unnecessary fetal and maternal harm. Last, since risk factors cited in this article such as drug, alcohol, and tobacco use are already routinely screened for in pregnant populations, new information concerning these factors could be of immediate practical significance. The goals of this study were accomplished by the confirmation of many interesting points, four of which I would like to comment on: (1) The authors reported a 20% prevalence of battering among women in their low-risk prenatal population. This high figure appears to be consistent with other reports of similar populations in the literature[1] and should serve to raise our consciousness about this probably grossly underreported problem. Patients in this study, however, were from a clinic setting and not from private offices. Can we extrapolate these results to society in general or do socioeconomic levels influence prevalence? (2) The investigators also showed an increased frequency of battering during pregnancy in 6% of cases and a decreased frequency of battering during pregnancy in 5% of cases. This appears to indicate no overall escalation of abuse during pregnancy as other studies claim. Furthermore, since no information was given regarding the onset of first battering, no conclusions can be drawn about whether pregnancy itself is a risk factor for the initiation of abuse. (3) Next, the manuscript stated that twice as many Anglo women as black or Hispanic women reported being physically abused. Does this represent greater reluctance on the part of black and Hispanic women to disclose this private and painful information for cultural reasons, is it simply a language miscommunication, or is it truly a difference in prevalence? Discussion should speculate about this finding. (4) Last, abused Hispanic women were more likely to be married than were nonabused Hispanic women. This is a reversal of the situation for Anglo and black women. It is difficult to interpret marital status as a risk factor, at least in Hispanics. Lower divorce rates are probably accounted for by the fact that the Hispanic population is largely Catholic.

The design of this study allowed for the collection of a large amount of data and the authors should be complimented on its detailed statistical analysis. I am uncertain, however, as to why patients under the age of 18 were excluded from the study sample and why only a low-risk population was queried. I also question the timing of patient interviews and why, as the authors commented, they chose not to delay questioning until the third trimester when a higher prevalence of abuse may have been detected. The addition of a table of demographic information including variables such as parity and socioeconomic status may serve to clarify the study population's characteristics.

Merit in this article lies in its ability to heighten awareness among health care providers of the very real and not so rare problem of physical abuse of the pregnant woman. Practically speaking, however, it is impossible, on the basis of the risk factors analyzed in this manuscript, to formulate the profile of a typical battered woman. The investigators have clearly demonstrated significant variations among the three racial groups studied. Fortunately, the habits of drug, alcohol, and tobacco use, as well as the history of an unstable marital relationship, in pregnant women usually alert clinicians to the need for closer pregnancy surveillance. Hopefully, this would lead to the discovery of an abuse problem if one existed. However, this is not enough. The screening of pregnant women must include some active assessment of their physical safety.

I would encourage these investigators to continue their work in this area, perhaps next sharing with us information regarding pregnancy outcomes in this series. It still remains to be seen whether identifying risk factors will actually prevent abuse.

Finally, I would like to draw attention to an excellent program funded by the March of Dimes Birth Defects Foundation designed to educate health care providers and the general public about battering during pregnancy, which was successful in the Houston area. Educational materials, as well as protocols of care for battered women, are available from this organization.

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Dr. Pedro A. Poma, Melrose Park, Illinois. I just want to underscore that spouse abuse may occur at any socioeconomic and educational levels. It occurs in most ethnic groups.

I also want to tell the authors that probably the word *Anglo* does not represent the ethnic background of the group they want to identify. In this country only a small percent have an Anglo background (i.e., people from England). A more appropriate name for such a group would be *non-Hispanic.*

Dr. Herbert F. Sandmire, Green Bay, Wisconsin. I would like to ask the authors who were the perpetrators—the other 20% who were not the spouse or boyfriend?

Dr. Daniel A. Rightmire, Springfield, Illinois. I have a number of comments. First, statistically speaking, gravidity, parity, and number of children are not continuous variables and should not be summarized by parametric statistics.

Second, it should be noted that a number of the variables are highly correlated and are not independent.

Another problem is that the study population was self-identified. Is there some other, more objective way of identifying physically abused persons? For example, creatine phosphokinase values might serve as an objective laboratory marker of physical abuse.

It is difficult to find a control or reference population or to quantitate the amount or frequency of violence in a person's life. Does the percentage of male spouses abused by pregnant women vary by race?

Dr. Washington C. Hill, Nashville, Tennessee. First, I congratulate the authors, particularly in viewing a subject and studying a subject that many of us are very reluctant to talk about.

I have two questions. The first is, since you took the time to look at this problem, I would like you to speculate on what you think you would find when you look at your private patients instead of your clinic patients. Also, what do you want to give as a take-home message to those of us who are seeing patients in our private practice?

Dr. Jacques F. Roux, Jackson, Wyoming. In Jackson, Wyoming, about 29% of women are possibly abused before pregnancy. They seem to accept abuse as a matter of fact. I wonder if this attitude could be related to their childhood experiences. For instance, a family member could have abused them sexually at an early age. This kind of data may reinforce the conclusions of this presentation.

President Malinak. I would like to ask Dr. Berenson a few questions. First, was the same question asked to those patients in your hospital who presented with no prenatal care? In other words, do you know the incidence of abuse in women who did not have prenatal care?

Second, were there differences in low-birth-weight infants in the abused versus nonabused groups of women?

Dr. Berenson (Closing). I would like to thank the discussants for their very kind remarks.

First, to answer Dr. Perkins' question, which comes first, drug abuse or battering? I don't think we have a definitive answer to that question, but I can speculate that women who use drugs are at higher risk of being abused on the basis of studies that demonstrated that children who were abused seemed to be at higher risk of using drugs. Other people who have done work in this field speculate that drug use may be an escape from both psychologic and physical pain as Dr. Perkins suggested.

It was also asked why we used patients over the age of 18, low-risk patients, and why they were screened at their second prenatal visit. You would have to understand a little bit about the University of Texas Medical Branch system, where I am from, to see why we set it up this way. This was a pilot screening program to detect physical abuse in our pregnant population, and the first clinic we set it up in was our low-risk prenatal clinic.

We have clinics in about 10 different counties, and we did not attempt to start screening every place at once. The second prenatal visit was chosen even though we realized it would miss some patients who were abused later in pregnancy because this allowed all women to be screened at least once. If we had chosen another time, for example, at 36 weeks' gestation, those who had a preterm delivery or an abortion might have been missed.

Can these results be applied to all women in our society? No, I don't think you can say that on the basis of this article. You have to keep in mind our population. We are screening women of childbearing age who are in a low socioeconomic status and who presented for prenatal care. We have not yet looked at some of the other groups that have been mentioned.

Is pregnancy itself a risk factor for abuse? We did find several patients who reported a decrease in abuse or an increase in abuse in pregnancy, but the numbers are too small to state that pregnancy was or was not a risk factor for abuse.

An important question was raised on whether Hispanic women underreport a history of battering. On the basis of anecdotal reports from our hospital, Hispanic patients are more reluctant to disclose physical abuse. More husbands of Hispanic women accompany their wives to the prenatal clinic and sometimes have to be asked to leave the room so that the survey can be conducted in private.

We are beginning to look at the outcome of the pregnancy in the first half of these patients that were screened, and to date we've found no difference in gestational age at the time of delivery or in Apgar scores. We plan to investigate this further.

I think the point is well taken that physical abuse occurs in families of all socioeconomic levels. We will probably never be able to determine the prevalence in a private population at our institution because we only have 10% of private patients.

I think the take-home message for those that work in clinics or private practice is that we need to screen our obstetric patients for a history of physical abuse. It's a very common event. It occurs in pregnancy, and we don't know whether the fetus is being placed at risk. We certainly will never know which patients are at risk if we don't ask.

Many of the women were very relieved to have someone just ask if something was going on. The reason we began screening was not primarily for research but to improve prenatal care and to educate women through counseling by a social worker.

It was pointed out that the variables are highly correlated, and I think that is true. We did attempt to adjust for age because we found that our drug-using population was older than our non-drug-using population, but many of these variables are highly correlated.

We did not investigate the men. We did not ask about their drug abuse or alcohol problems, and we do not know their races.

I hope this answers all of the questions.

The natural history of HELLP syndrome: Patterns of disease progression and regression

James N. Martin, Jr., MD, Pamela G. Blake, MSN, Kenneth G. Perry, Jr., MD,
James F. McCaul, MD, L. Wayne Hess, MD, and Rick W. Martin, MD
Jackson, Mississippi

Despite much recent interest in HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count), there is little published information about the natural history of this unique form of severe preeclampsia-eclampsia. The time course and pattern of laboratory abnormalities for 158 patients with HELLP syndrome managed in a single tertiary referral center between 1980 and 1989 were studied retrospectively. Despite considerable patient variation, most gravid women with HELLP syndrome had decreasing platelet counts until 24 to 48 hours after delivery. Conversely, lactate dehydrogenase concentrations usually peaked 24 to 48 hours post partum. In all patients who recovered, a platelet count $>1,000,000/mm^3$ was spontaneously achieved by the sixth postpartum day or within 72 hours of platelet nadir. An upward trend in platelet count and a downward trend in lactate dehydrogenase concentrations should be apparent in patients without complications by the fourth postpartum day. These data provide baseline information against which the course of individual patients can be compared and the infrequent, atypical case identified for interventive therapy. (AM J OBSTET GYNECOL 1991;164:1500-13.)

Key words: Severe preeclampsia-eclampsia, HELLP syndrome, natural history

HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) is a multisystem disease form of severe preeclampsia-eclampsia that is characterized by microangiopathic hemolytic anemia, hepatic dysfunction, and thrombocytopenia and that, in most severe cases, progresses to disseminated intravascular coagulation.[1-8] Insidious in onset, obtuse to easy clinical recognition, and often misinterpreted as a nonobstetric disorder, HELLP syndrome affects an estimated 4% to 12% of patients with severe preeclampsia.[9-13] Recognition of its presence usually is triggered by clinical signs and symptoms of hepatic involvement such as epigastric pain, hepatic or right upper-quadrant tenderness, nausea, and malaise. Because HELLP syndrome often is not attended by the usual signs and symptoms of its parent disease preeclampsia (severe hypertension, proteinuria), in its earlier, milder form it can be missed unless appropriate laboratory assessment is performed by an alert clinician. Thus the HELLP process is often advanced before an accurate diagnosis is made, rendering it difficult to identify patients for study early in the course of the disorder.

Presently there are only two large published studies of patients with HELLP syndrome.[4,9] In spite of these data, there is a paucity of information regarding the natural history of this unique pregnancy-associated condition, especially in relation to patterns of laboratory parameters observed during the peripartal course.

The purpose of this report is to communicate the findings from a large population of patients with HELLP syndrome specifically related to the natural history of this disorder as reflected by the pattern and time course of laboratory abnormalities.

Patients and methods

A computer search of the medical records of all obstetric patients delivered at the University of Mississippi Medical Center between Jan. 1, 1980, and March 31, 1989, was undertaken to identify all patients with HELLP syndrome. Inclusion criteria were as follows: (1) thrombocytopenia defined as a peripartal platelet concentration $\leq 100,000/mm^3$ associated with a normal platelet count documented prenatally or during the postpartum period; (2) evidence of hepatic dysfunction to include an increase (mean ± 3 SD above third-trimester normal values) in aspartate aminotransferase (≥ 48 IU/L), alanine aminotransferase (≥ 24 IU/L), or lactate dehydrogenase (≥ 164 IU/L); (3) evidence of intravascular hemolysis to include a decreas-

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ing hematocrit concurrent with worsening thrombocytopenia, an increase in lactate dehydrogenase, and/or evidence of bleeding involving gums, intravenous sites, or hematuria; (4) no evidence of another disorder primarily causative of the laboratory and clinical findings.

During the 111-month period, 158 pregnancies met inclusion criteria for HELLP syndrome. All pregnancies were subclassified into one of two classes of HELLP syndrome on the basis of only the lowest platelet count recorded at any time during the patient's peripartal course. In patients with class 1 HELLP syndrome platelet counts fell ≤50,000/mm^3; class 2 HELLP syndrome includes patients whose platelet nadir occurred in the range of >50,000/mm^3 to ≤100,000/mm^3.

The hospital chart for each affected pregnancy in this study was reviewed extensively, and pertinent data were entered into a computerized relational data base before statistical analysis. The two classes of HELLP syndrome (classes 1 and 2) considered in this portion of a larger ongoing group of investigations of the entire study population were compared by χ^2 analysis for categoric data and analysis of variance for continuous data; a difference was considered significant at $p < 0.05$.

Results

Inclusion criteria for this investigation were satisfied by 158 gravid women including 92 primigravid (58.2%) and 66 multiparous (41.8%) women. Black patients constituted the majority (110 or 69.6%) of patients with HELLP in both class groups; additionally there were 39 white patients and 9 native American patients. Mean age (±SD) of all patients was 22.9 ± 5.5 years with a range of 14 to 42 years. The mean weight (±SD) for all patients was 161 ± 34.7 pounds and mean height (±SD) was 63.8 ± 3 inches. There were no significant differences between the class 1 and 2 HELLP patient groups.

The length of time between hospital admission and delivery ranged between 1 and 61 hours in the class 1 patient group with a mean interval (±SD) of 11.4 ± 11 hours; only 4 of 70 patients (5.7%) remained undelivered >24 hours in this group. Analysis of the data for class 2 patients revealed an admission-to-delivery time interval of 2 to 81 hours with a mean (±SD) of 15.6 ± 15.3 hours; it exceeded 24 hours in only 12 of 88 patients (13.6%).

At the time of hospital admission, only 24 of 70 pregnancies (34%) eventually determined to have class 1 HELLP syndrome had a platelet count ≤50,000/mm^3 (Fig. 1, top). Later, at delivery, 33 of 68 evaluable pregnancies (48.5%) had a fall to this platelet concentration. The highest percentage of class 1 patients with a platelet concentration ≤50,000/mm^3 (65.6%) occurred on the first postpartum day. By the sixth postpartum day only 6 (6.6%) class 1 patients remained in the ≤50,000/mm^3 range. All recovered by the eleventh postpartum day. The platelet nadir for patients in class 1 occurred on admission in 9 patients (13%), at delivery in 20 (29%), on postpartum day 1 in 21 (30%), on postpartum day 2 in 15 (21%), on postpartum day 3 in 2 patients, on postpartum day 5 in 2 patients, and on day 7 in one late postpartum patient with eclampsia (Fig. 1, bottom).

Data for pregnancies later classified as class 2 HELLP syndrome were similar to those for class 1. Only 26 of 88 pregnancies (30%), when patients were first admitted to the hospital, had a platelet count in the class 2 range of >50,000/mm^3 to 100,000/mm^3 (Fig. 2, top). At delivery 48 of 88 pregnancies (54.5%) were within the final HELLP classification range. The highest percentages of class 2 patients within a platelet concentration of >50,000/mm^3 to 100,000/mm^3 occurred on postpartum days 1 (69.2%) and 2 (70.8%). By postpartum day 6 all class 2 HELLP pregnancies exceeded a platelet concentration of ≥100,000/mm^3. The platelet nadir for patients in class 2 occurred on admission in 8 patients (9%), at delivery in 19 patients (21%), on postpartum day 1 in 32 (37%), on postpartum day 2 in 23 (26%), and on postpartum day 3 in 6 (7%) (Fig. 2, bottom). Combined data for all patients in classes 1 and 2 including all recorded platelet measurements are depicted in Fig. 3.

The rate of platelet decrease per day between hospital admission and delivery was determined in 139 of the 158 combined patients from classes 1 and 2; in 19 others (9 in class 1, 10 in class 2) a platelet nadir was achieved at hospital admission and increased (primarily as a result of platelet transfusion in 14 of the 19 patients) or remained relatively unchanged thereafter (Fig. 4). At the time of hospital admission, patients with HELLP syndrome fell into one of four ranges of platelet concentration. First, 30 of 158 patients (19%) were admitted with a normal platelet concentration (>150,000/mm^3). Between admission and delivery, in half of this group, a 40% decrease in platelet concentration within the normal range was demonstrated. In the other 15 patients a mean platelet decrease of 43% to concentrations between 100,000/mm^3 and 150,000/mm^3 (9 patients) or between 50,000/mm^3 and 100,000/mm^3 (6 patients) was demonstrated. Second, in 55 of 158 patients (35%) concentrations ranged between >100,000/mm^3 to ≤150,000/mm^3 at the time of hospital admission; in 34 patients platelet counts decreased 24% to remain within the same platelet range, in 18 (28%) they decreased to the >50,000/mm^3 to ≤100,000/mm^3 range, and platelet concentrations of 3 patients decreased 53% to <50,000/mm^3. Third, 49 patients (31%) were admitted with a platelet range be-

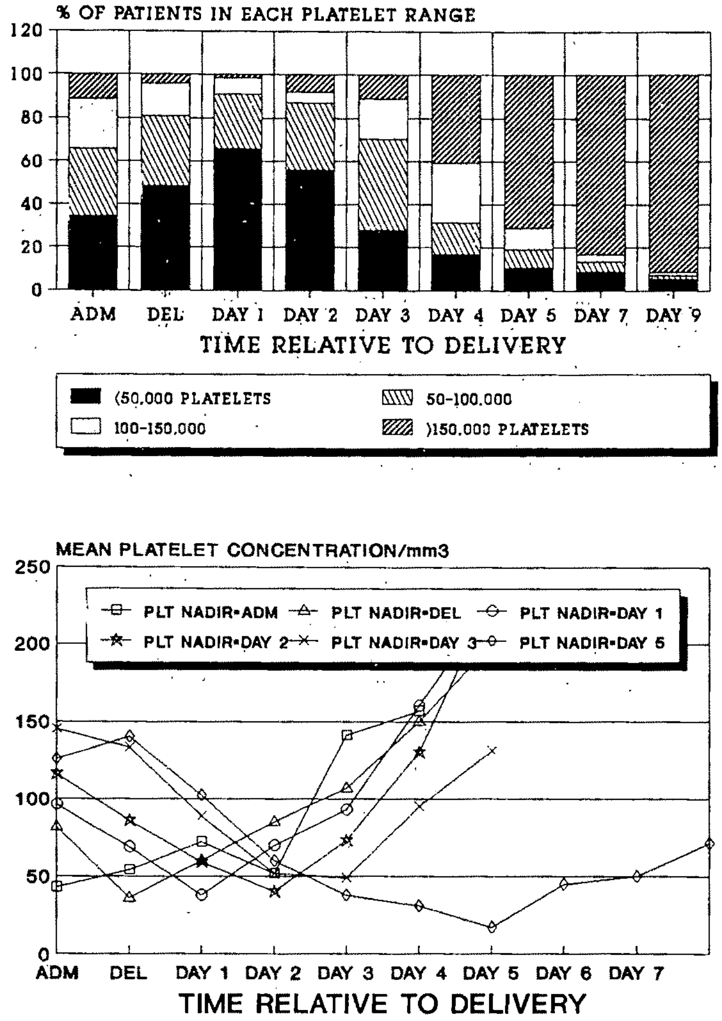

Fig. 1. Range of distribution of platelet counts at various intervals before and after delivery during clinical courses of 70 pregnancies with class 1 HELLP syndrome *(top)* and platelet means for groups of patients with platelet nadirs on different peripartal days *(bottom)*.

tween >50,000/mm^3 and ≤100,000/mm^3; in 29 concentrations remained within this range with an average 23% decrease and in 10 concentrations fell an average of 51% to <50,000/mm^3. In 10 other patients (5 were transfused) a 20% increase was demonstrated within the same range with only one value in excess of 100,000/mm^3 platelets. Finally, 24 patients (15%) with HELLP syndrome were admitted with platelet values ≤50,000/mm^3; values decreased in 13 (36%) before delivery whereas in 2 other patients they decreased 21% and then increased, along with 9 others (transfused), into the 50,000/mm^3 to 100,000/mm^3 range at the time of delivery (Fig. 4).

The percentage of patients in each of the two HELLP classes that exceeded a lactate dehydrogenase serum concentration of 600 IU/L was plotted for each day relative to time of delivery (Fig. 5). This figure was exceeded in the largest numbers of patients on postpartum day 2 for class 1 patients (64%) and on postpartum day 1 for class 2 patients (31%). Thereafter, there was a downward trend in both classes with exceptional persistent elevations or rising concentrations in the few patients in class 1 who eventually underwent plasma exchange therapy. An inverse relationship between the trends in platelet values and lactate dehydrogenase concentrations was seen in both classes of HELLP patients (Fig. 6). Serum concentrations of aspartate aminotransferase and alanine aminotransferase generally paralleled lactate dehydrogenase during the course of HELLP syndrome but at comparatively much reduced serum concentrations as illustrated in Fig. 7. Of the two tests of liver function, aspartate aminotransferase was generally higher than alanine aminotransferase during the acute phase of the disease, around delivery. At this time the largest difference between the two values was apparent. During recovery the aspartate

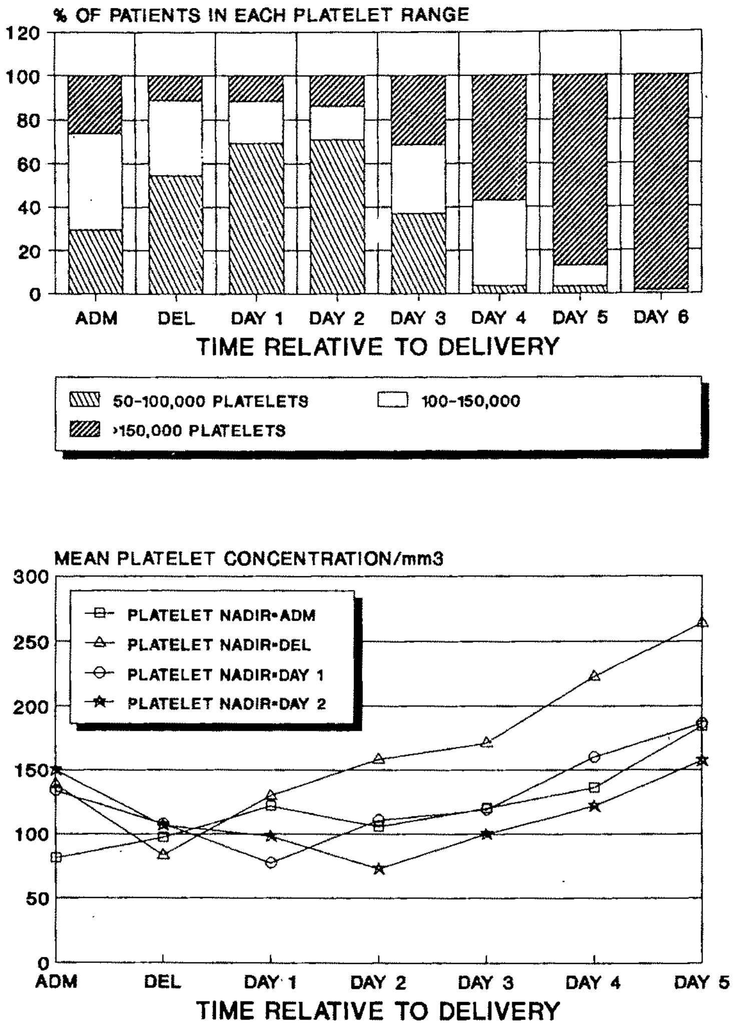

Fig. 2. Range of distribution of platelet counts at various intervals before and after delivery during clinical courses of 88 pregnancies with class 2 HELLP syndrome *(top)* and platelet means for groups of patients with platelet nadirs on various peripartal days *(bottom)*.

aminotransferase/alanine aminotransferase ratio decreased and eventually reversed or reached parity.

The relationship between mean hematocrit and platelet concentration relative to time of delivery in class 1 and 2 patients who did not receive red blood cell or platelet transfusions is shown in Fig. 8. A downward trend in hematocrit after admission and delivery generally continued until the second (class 1) or third (class 2) postpartum day.

Eleven patients in the class 1 study group underwent plasma exchange therapy. Four were treated in the immediate peripartal period (±24 hours of delivery) for unusually severe disease manifestations, including worsening severe thrombocytopenia with clinically evident bleeding, persistent clinical deterioration despite conventional transfusion therapy, and worsening neurologic status with concern for intracranial bleeding. The other 7 patients underwent exchange between the third and ninth postpartum day as described in detail recently.[14] All of the 7 patients but one had postpartum evidence of persistent or worsening severe thrombocytopenia in association with rising lactate dehydrogenase concentrations. The single exception was a patient that failed to show evidence of HELLP syndrome until the sixth postpartum day after an eclamptic seizure.

Comment

The patient population presented in this investigation represents the largest single group of patients with HELLP syndrome found in the obstetric literature. The most important finding of this study is the demonstration of the dynamics of this disease process as reflected in constantly changing laboratory parameters and the demonstration of how the patterns of disease progression and regression occur at variable time intervals in

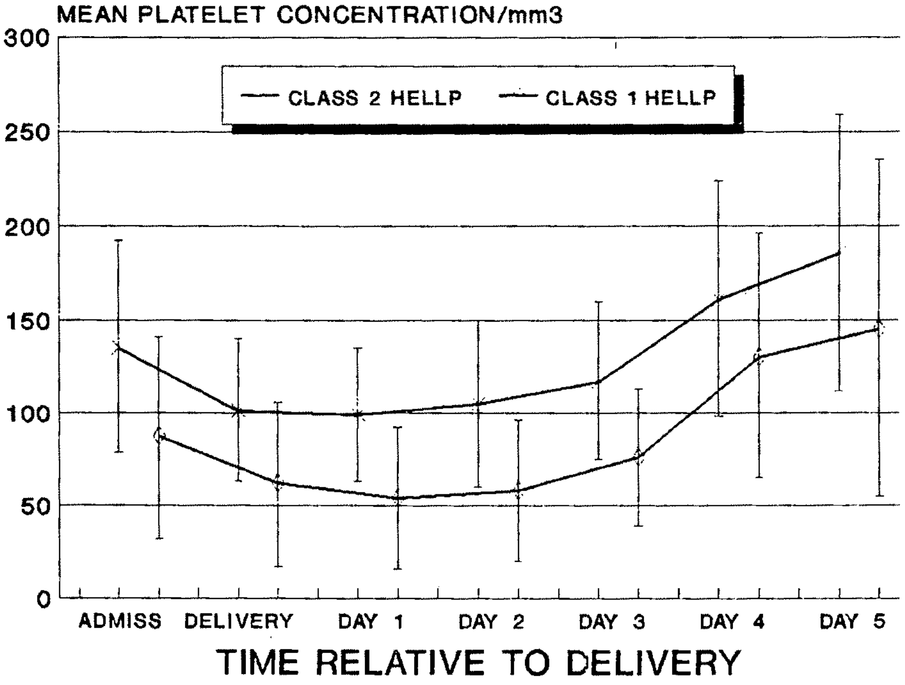

Fig. 3. Plotted platelet count means for all class 1 and class 2 HELLP pregnancies with platelet nadirs on day of hospital admission, day of delivery, or postpartum day.

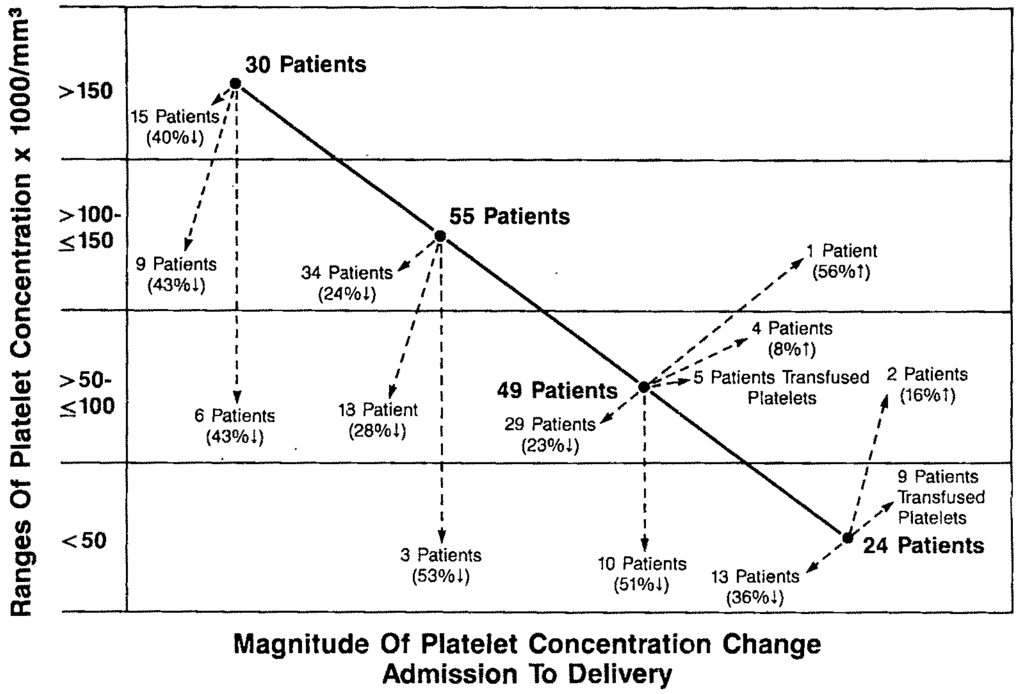

Fig. 4. Direction and degree of change in platelet concentration for 158 patients with HELLP syndrome, plotted between hospital admission and delivery.

HELLP syndrome. The disease appears to achieve peak intensity during the 24 to 48 hours after delivery. How rapidly a patient recovers from HELLP-complicated preeclamptic pregnancies depends on a number of factors, including when in the course of disease progression the pregnancy termination occurs. A small subset of patients appear to have such severe multisystemic disease that even discontinuation of pregnancy is in-

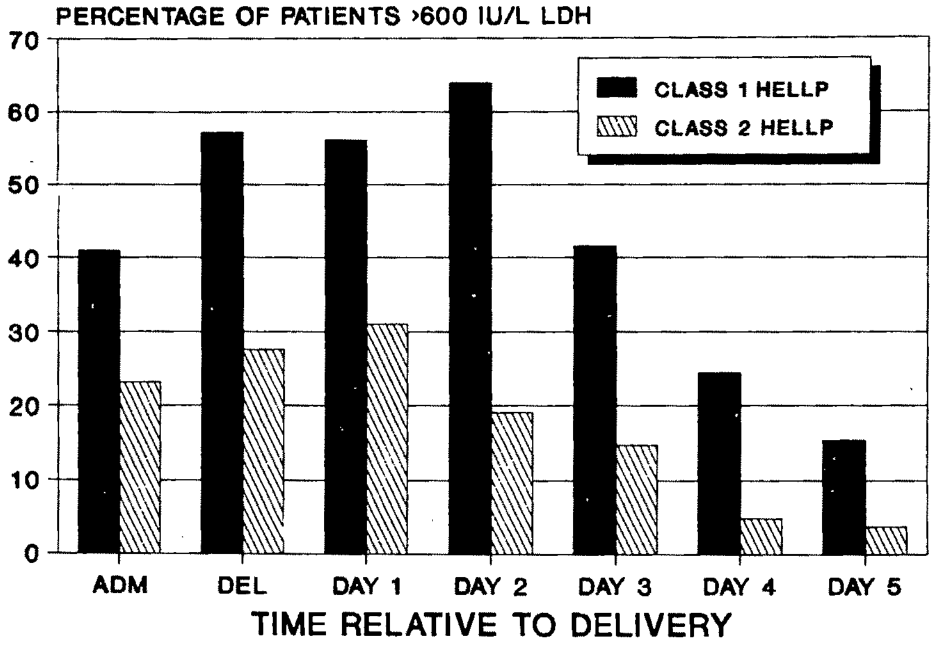

Fig. 5. Percentages of patients in class 1 and class 2 HELLP syndrome each day with lactate dehydrogenase values > 600 IU/L.

adequate to ensure timely recovery, if at all, and other treatment such as plasma exchange is necessary.[4, 15-19]

Because of the dynamic natural history of HELLP syndrome, prospective attempts at disease subclassification with prediction of eventual disease severity is a frustrating undertaking. The retrospective classification system used for the analysis of patients in the Mississippi study population has been utilized to standardize evaluation of these patients and permit comparative assessment of a number of parameters pertinent to clinical management concerns. Recently, Van Dam et al.[20] reported laboratory findings related to the development of disseminated intravascular coagulation in 18 patients with HELLP syndrome. Interestingly, the first two of their three categories of "manifest," "suspected," and "no evidence of" disseminated intravascular coagulation compare closely with the platelet counts used retrospectively to classify patients in this investigation as having class 1 or 2 HELLP syndrome.

Three other groups of investigators also have attempted to plot the course of thrombocytopenia and other laboratory parameters in the course of peripartal HELLP syndrome. Stedman et al.[21] in Birmingham evaluated 12 patients with severe preeclampsia or eclampsia and coincident HELLP syndrome during and after delivery. All patients were delivered within 24 hours of admission, and laboratory measurements were continued until test results returned to normal values. Patients in this study had a mixture of class 1 and class 2 disease with platelet nadirs between 22,000/mm³ and 55,000/mm³. The lowest observed platelet count occurred at a mean of 23 hours postpartum, a result very similar to that of this investigation. The mean maximal aspartate aminotransferase level of 694 IU/L was 17 times the upper limit of normal. There was a return of aspartate aminotransferase toward normal values within 3 to 5 days after delivery. Maximal lactate dehydrogenase levels ranged from 581 to 2380 IU/L with a mean of 1369 IU/L. In 7 of 10 patients lactate dehydrogenase values exceeded 900 IU/L.

Catanzarite[22] retrospectively reviewed the perinatal course of 79 women with HELLP syndrome whose platelet counts fell below 80,000/mm³. This investigator also found that the lowest platelet count did not predict peak values of aspartate aminotransferase or lactate dehydrogenase and that the peak alanine aminotransferase was lower than the peak aspartate aminotransferase with a mean ratio of 0.55. The degree of abnormality of platelet count, aspartate aminotransferase, and lactate dehydrogenase did not prospectively and accurately predict time to recovery. Consistent with the findings in this report, Catanzarite observed that the lowest platelet count and mean aspartate aminotransferase and lactate dehydrogenase occurred within 72 hours of delivery in almost all cases. At 5 and 7 days after delivery, platelet counts were above 100,000/mm³ in 85% and 96% of his cases, respectively. Aspartate aminotransferase values were below 70 IU/L in 85% and 96% and lactate dehydrogenase values were below 400 IU/L in 76% and 89% at 5 and 7 days, respectively.

Neiger et al.[23] prospectively followed up 20 patients with class 1 and 2 HELLP syndrome. The mean platelet nadir for the group was 50,000/mm³ and the

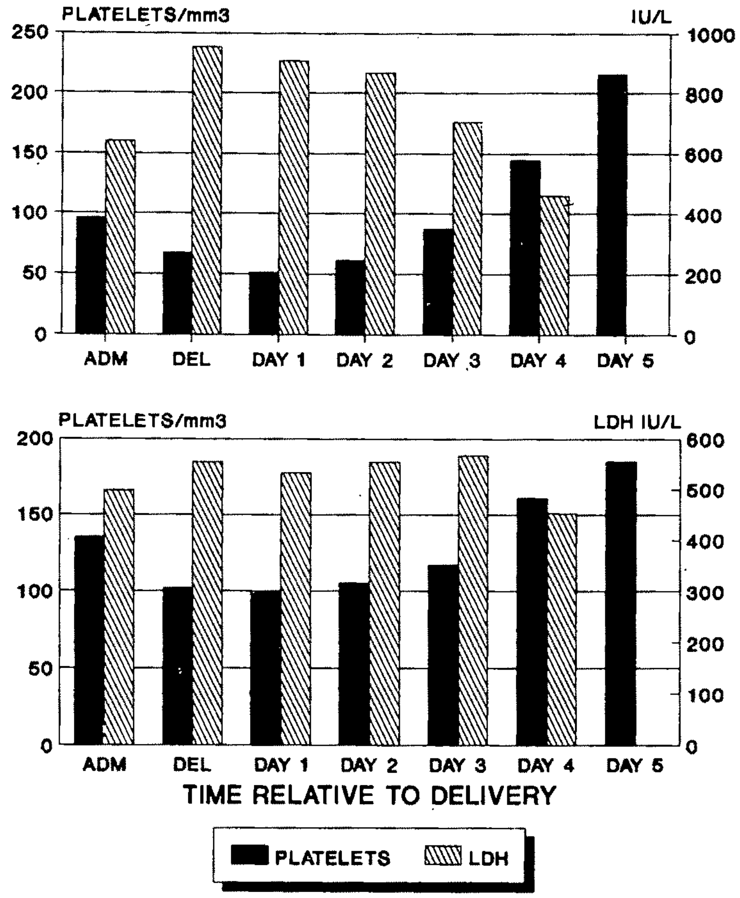

Fig. 6. Daily mean platelet counts and lactate dehydrogenase serum concentrations from admission through fifth postpartum day, plotted with *bars* for pattern comparison in class 1 *(top)* and class 2 *(bottom)*.

average time from delivery to platelet count recovery >100,00/mm^3 was 82 hours. The value was somewhat skewed because two patients required 140 and 270 hours for spontaneous platelet recovery whereas the other 18 recovered by the fifth postpartum day.

Although several investigators have attempted to define the parameters of recovery for patients with HELLP syndrome, no information to date is available regarding the rapidity with which the disease worsens. In this investigation the retrospective data suggest that at least 88% of patients with either class 1 or 2 HELLP syndrome experience worsening thrombocytopenia until and for many hours after delivery. Regardless of whether the platelet count at the time of hospital admission is in the normal range or as low as <50,000/mm^3, patients in both HELLP classes usually showed evidence of a decreasing platelet count at the average rate of approximately 40% per day. A downward trend in platelet count and an upward trend in lactate dehydrogenase and transaminase serum concentrations persists in most patients into the second postpartum day before a trend toward normalization begins. The line plots in Fig. 3 show that the platelet count should be expected to exceed the 100,000/mm^3 level no longer than 72 hours after platelet nadir (*not* delivery) occurs, unless the patient has severe, atypical disease. Stated another way, an upward trend in platelet count should be apparent by the fourth postpartum day. Despite the limitations of the retrospective nature of this investigation, it has been demonstrated that a return to normal laboratory parameters appears to be dependent largely on when laboratory nadirs occur for the various tests of dysfunction in HELLP syndrome.

It appears that patients with class 1 HELLP syndrome require a longer interval of time for complete resolution of thrombocytopenia, hemolysis, and liver dysfunction. Although all patients with class 2 HELLP had an increase in platelet counts to >100,000/mm^3 by the sixth postpartum day, a small number of patients in class 1 had persistence in the <50,000/mm^3 range for several days thereafter, until plasma exchange was undertaken. In this patient series it was not until the eleventh postpartum day that all patients in class 1

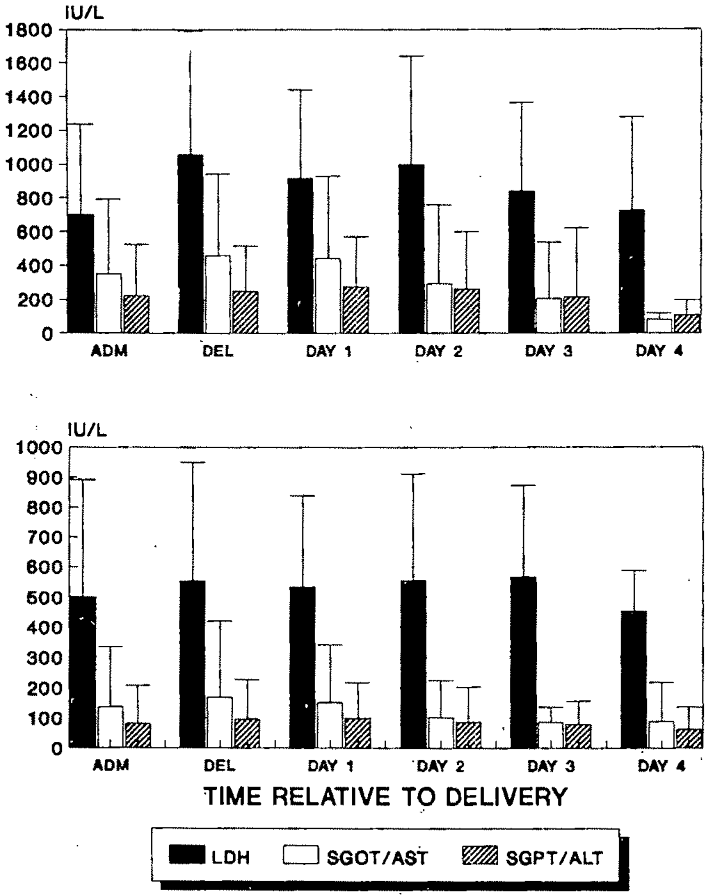

Fig. 7. Mean values of lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase for all patients with class 1 HELLP syndrome *(top)* and class 2 HELLP syndrome *(bottom)*, plotted from hospital admission through fourth postpartum day.

had a return with assistance to a platelet concentration $>100,000/mm^3$.

The difference between class 1 and class 2 platelet normalization patterns is primarily accounted for by a small number of patients who failed to recover spontaneously and instead had persistence of severe atypical systemic disease. Seven patients in this series underwent single or repetitive plasma exchange procedures beyond 72 hours post partum in an effort to effect arrest and reversal of the HELLP process.[14] In an earlier published study of the 7 patients who underwent plasma exchange, it was emphasized that a trend of rising lactate dehydrogenase values in combination with a persistent or decreasing platelet count $<500,000/mm^3$ >72 hours postpartum usually represents atypical HELLP syndrome that will be responsive to plasma exchange therapy.[14]

Of all the potential, commonly available tests of liver dysfunction for the assessment and management of patients with HELLP syndrome, lactate dehydrogenase reliably demonstrates the most dramatic rise in serum levels. As a reflector of degree of hemolysis, as well as hepatic dysfunction, lactate dehydrogenase concentrations usually peak on the first postpartum day in class 2 patients and by the second postpartum day in class 1 patients. Mean values of platelets and lactate dehydrogenase for class 1 patients suggest that the downward trend in lactate dehydrogenase values begins at approximately the same time that the upward trend begins with platelet concentrations. Over time, lactate dehydrogenase values tend to be inversely proportional to the trend in platelet counts (Fig. 6). Such a trend and pattern are not as clear in the accumulated data from the less severely affected class 2 HELLP patients. In this latter group increased lactate dehydrogenase values appear to persist for a longer interval at a lower level than is the case with class 1.

The use of lactate dehydrogenase values in excess of either 500 or 600 IU/L as an index of disease severity is an arbitrary decision. The former value is utilized by hematologists at this institution for the monitoring of patients with thrombotic thrombocytopenic purpura, and the latter value is adapted from work by Sibai.[21] The percentage of patients with LDH concentra-

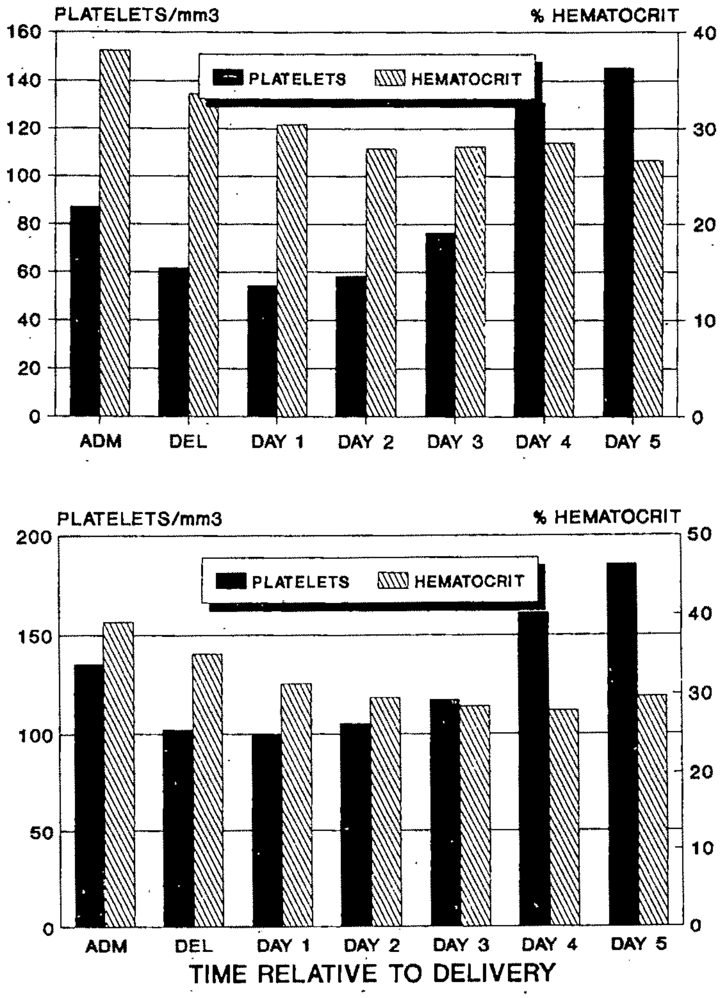

Fig. 8. Relationship between mean values of platelet count and hematocrit for all patients with class 1 HELLP syndrome *(top)* and class 2 HELLP syndrome *(bottom)*, demonstrated from hospital admission through fifth postpartum day.

tions > 600 IU/L on each peripartal day as illustrated in Fig. 5 appears to parallel the course of HELLP syndrome severity and could be a useful adjunct to patient management. Only patients who required plasma exchange therapy persisted in having lactate dehydrogenase concentrations in excess of 600 IU/L beyond the fourth postpartum day.

Aspartate aminotransferase appears to be the dominant transaminase released into the peripheral circulation with severe preeclampsia and HELLP syndrome.[4, 9, 26] Similarly, Catanzarite[22] observed a peak alanine aminotransferase/peak aspartate aminotransferase ratio of 0.55 in his series of 23 patients with class 1 and 2 HELLP. Not until late in the puerperium did serum concentrations of alanine aminotransferase exceed aspartate aminostransferase values in the present investigation. Importantly, high aspartate aminotransferase/alanine aminotransferase ratios were observed during the most active phases of the HELLP syndrome process. The dominance of aspartate aminotransferase relative to alanine aminotransferase was lost, and eventually the ratio reversed during the recovery phase of the disease.

Although microangiopathic hemolytic anemia underlies HELLP syndrome, paradoxically most patients are not anemic when first admitted to the hospital. The highest recorded mean hematocrit in this investigation was observed at hospital admission for both groups of patients. In both class 1 and 2 patient groups there was generally a downward trend in hematocrit after admission until the second postpartum day, after which a plateau effect was observed. It appears that hemolysis with secondary anemia is either arrested or effectively neutralized by hematopoiesis and red blood cell release, usually within 48 hours of delivery for patients with uncomplicated HELLP syndrome.

The natural history of HELLP syndrome as reflected by a variety of laboratory parameters has revealed its variety of presentation yet its relative predictability. The retrospective nature of this investigation precludes more definite hourly rates of rise and fall for laboratory parameters. Extensive prospective investigations of patients with HELLP syndrome could afford more definite assessment of disease status and improve guidance of therapeutic interventions so that the adverse maternal[4, 9] and perinatal[4, 9] effects (unpublished observations) of this "great masquerade" can be averted or minimized.

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Discussion

DR. NORBERT GLEICHER, Chicago, Illinois. The article by Martin et al. represents a timely contribution to a still poorly understood clinical entity, initially described by Weinstein[1] in an astute clinical observation. Ever since, and once again in this article, the HELLP syndrome has been characterized as a multisystem disease form of severe preeclampsia-eclampsia. If one accepts this premise, then the HELLP syndrome may, in fact, contribute to our understanding of preeclampsia-eclampsia.

Martin et al. summarized 158 gravid women, after a retroactive review of patient charts for a period of 9 years 3 months, who qualified as HELLP patients. Whereas any such *retroactive* chart review has obvious shortcomings and has to represent a selection bias toward more severe cases, this nevertheless represents the largest number of patients with HELLP so far reported in the literature in a single study. In that aspect alone it represents a unique resource.

I would like to utilize this resource in attempting to point out two very important observations that did not receive the deserved attention in the authors' discussion. Both may, in my opinion, shed light on the pathophysiologic characteristics of preeclampsia-eclampsia. First, I was pleased to note that an unexpectantly high percentage of affected women were multiparous

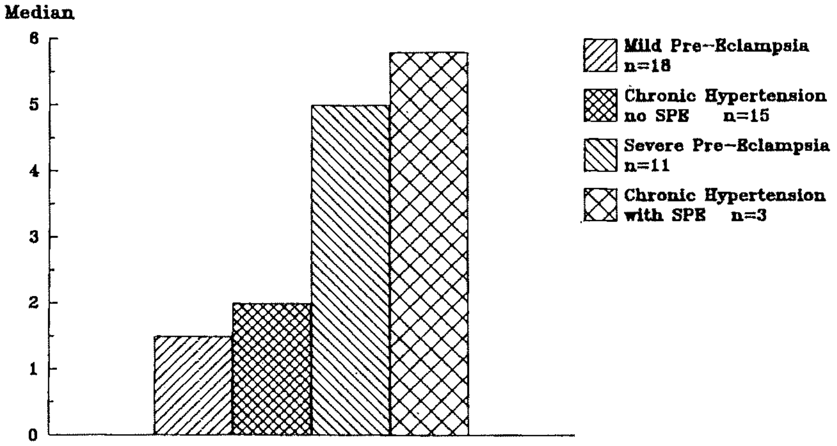

Fig. 1. Autoantibody positivities in hypertensive conditions of pregnancy.

(42%). On first impulse this should surprise, since preeclampsia-eclampsia is generally considered a disease of the primiparous woman. Second, the authors reported that HELLP abnormalities, such as thrombocytopenia and liver abnormalities, peak at 24 to 48 hours *after* delivery. This once again does *not* correspond with the reported classic natural history of preeclampsia-eclampsia, which suggests a return to normal within 24 hours from delivery.

What then can explain these observations? Martin and associates reported in an earlier publication that plasmapheresis improves HELLP parameters in otherwise nonresponsive patients in the postpartum period.[2] Other reports confirm this and also suggest a beneficial effect in the antepartum period, as so well reviewed recently by Watson et al.[3] Whereas plasmapheresis can remove a large variety of biologically active substances, there are reasons to believe that the culprits in HELLP are antibodies. Plasmapheresis is highly efficient in the removal of antibodies.[3] Antibodies to platelets have been reported in women with preeclampsia-associated thrombocytopenia.[4] Moreover, we[5] and others[6] have recently reported significant autoantibody abnormalities, and especially antiphospholipid antibodies, in patients with hypertensive diseases of pregnancy. Fig. 1 demonstrates that the number of autoantibody abnormalities increases with severity of maternal disease, if maternal disease is classified in accordance with Chesley's classification,[7] which correlates with adverse perinatal outcome. This figure also demonstrates that autoantibody abnormalities are not exclusively restricted to classically primiparous women with preeclampsia but also can be found frequently in multiparous women with chronic forms of hypertension. Therefore it was not surprising that Martin et al. reported such an unusually high incidence of multiparous patients among their HELLP population.

This then raises the question of from where the stimulating epitopes are derived. Once again, the study presented here may suggest an answer. The report that HELLP peaks 24 to 28 hours *after* delivery suggests that the causative epitopes reach maximal levels at or around the time of delivery. This is also the time period of largest fetomaternal cell "traffic." In an earlier presentation to this society, we have proposed that pregnancy be understood not only as an allograft but also as an autograft.[8] Maternal responses to self-components, derived from the fetus by fetomaternal cell "traffic," result in autoantibody production, which, if excessive, causes clinical autoimmune phenomena as seen in HELLP.

Under such a concept, HELLP is, in fact, an extreme form of preeclampsia-eclampsia but not necessarily in the traditional sense. Most likely, the patient with HELLP is extreme in either fetomaternal cell traffic or in genetic susceptibility to antigen self-stimulation, a concept also supported by recent reports of nonhypertensive HELLP. Last, under such a concept HELLP is only one among many *peripartal autoimmune syndromes,* characterized by peripartal exacerbation.

The authors have once again demonstrated that the investigation of extremes can further our understanding of the common in such poorly understood clinical situations as preeclampsia-eclampsia.

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DR. STEPHEN S. ENTMAN, Nashville, Tennessee. I am delighted to see Dr. Martin and his colleagues present these data that further clarify this syndrome of multisystem organ failure. This article is important for two reasons. It expands our knowledge. Since Dr. Pritchard first alerted us to the hemolytic manifestations, we have tried to understand how to place the pieces into the practical puzzle of clinical care. Popularized as the HELLP syndrome, the diagnosis is now more frequently, consistently, and appropriately made. Dr. Martin and associates, in this work and in a previously published article on plasma exchange,[1] have begun to answer some of the important practical clinical questions.

The second reason that this article is important relates to the philosophy of how new techniques and technology are introduced into the literature and then into the practice setting. These are the data that provide the foundation to support the use of plasma exchange therapy in the postpartum patient. In contrast to other popularized techniques, there is a data base available on which to form judgments about who, when, what, and why to subject patients to the risk and expense of a procedure. All too often, we have had to retroactively fit the foundation of data to justify diagnostic or therapeutic procedures that have already entered the practice of medicine. A purist might prefer that this article have anteceded the one promoting postpartum plasma exchange, published in January 1990, it is clear that the authors understood the content of these data in formulating their previous work. Now, without having been prompted by criticism, they have brought it to the rest of us. For this approach, they are to be commended.

After reading the entire manuscript, I do have several points to raise and several questions of Dr. Martin. Would you explain the rationale for separating patients into class 1 and class 2? It seems to me that this is an artificial division of continuously distributed platelet data into dichotomous data. This is especially concerning with retrospective data that may or may not have been obtained at the peak of the clinical manifestations or at the nadir of the platelet count. Platelet counts reflect a continuum of severity of the syndrome. In fact, there is only minimal difference between the two groups in the shape of the curve illustrating time course to recovery. I would prefer that you highlight those patients who ended up with transfusion or plasma exchange. These are, in fact, the patients whom you are most interested in distinguishing because their clinical course was atypical. Your goal in educating us is to help us understand the criteria for intervention.

Regarding the demographics of the study group, in comparison with the rest of your patient base and those patients with pregnancy-induced hypertension who did not have HELLP, was the racial, maternal age, gestational age, and parity distribution of these patients noteworthy? Are you confident that all patients with this syndrome were identified? I raise this question because you report an average of only 20 cases per year; in contrast, in our institution we have 50 to 60 cases per year. This may be a function of the difference between criteria for a clinical diagnosis compared with those for a diagnosis for investigative purposes. Specifically, I refer to inclusion criterion number 3, which demands overt evidence of gross hemolysis, evidenced by falling hematocrit, or overt coagulopathy, evidenced by abnormal bleeding. In our experience these patients are uncommon. We have always found it difficult to assess hematocrit changes in the face of hemoconcentration, intravenous fluid replacement, peripartal bleeding, and subsequent fluid shift during recovery. I wonder if you have a sense of the frequency with which your patients with a clinical diagnosis of HELLP have not met the study criteria.

I would caution the authors to review the distribution of their data for enzymes. It is a common pitfall to apply parametric statistics to enzyme data, but the gross deviation of the data curve from normalcy results in quite misleading information. Analysis of variance tests the distribution of data but assumes comparable variance in the comparison groups. A nonparametric test such as the Mann-Whitney U test might be more appropriate since it makes no assumptions about the shape of the data curve.

I have a further question about the data on elevated enzymes. In the manuscript there was significant attention to the shifting ratio of aspartate aminotransferase/alanine aminotransferase. Could you elaborate on the implications of that shift? Also, although I recognize that these data were compiled retrospectively, I wonder if you have any information about lactate dehydrogenase isozymes.

In summary, this report contains important information for those of us practicing high-risk obstetrics. It helps us distinguish those patients who will have spontaneous recovery from those who will benefit from therapeutic intervention. Understanding this will have a positive effect on the health of perinatal patients.

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DR. CHARLES A. STEDMAN, New Orleans, Louisiana. Did you have the opportunity to separate out the patients who had undergone cesarean section versus those who were delivered vaginally to see if there was any difference in the recovery phase?

Dr. Richard P. Perkins, Omaha, Nebraska. I basically wanted to ask the same question that Dr. Stedman did. Our own studies followed these same results pretty closely. I think it is hazardous to try to determine the natural history of a platelet count in a patient who has had a massive tissue injury, such as cesarean delivery. I would be interested in what your cesarean section rate was and whether there was any difference between the cesarean and vaginal delivery populations.

In our own series this disease occurs at a stage in the pregnancy where about 86% of women are delivered abdominally, and that doesn't leave very many patients for a true study of the natural history. I would also ask whether patients were excluded or otherwise accounted for who had, in fact, received platelet transfusions before the performance of cesarean section, if indeed you do those.

Dr. Carolyn B. Coulam, Indianapolis, Indiana. Like Dr. Gleicher, I'm intrigued with the multiparous population with HELLP syndrome and have three questions in that regard for Dr. Martin.

(1) How many patients in your series were multiparous? (2) Of the multiparous population, how many of these women had had previous pregnancies with HELLP syndrome? (3) How many of these multiparous patients were with the same partner that they previously had?

Dr. Paul G. Tomich, Maywood, Illinois. With regard to the thrombocytopenia, what percentage of patients in class 1 and class 2 had platelet transfusions? Were they predelivery or postdelivery transfusions?

Dr. Martin (Closing). I thank all the discussants for their questions. You will have to invite me back perhaps for some of the answers.

The first discussant, Dr. Gleicher, raised some questions about the immunology of this disorder. Certainly that is something that's been discussed by many people. An alternate explanation for the findings seen might be that they result from the ongoing reparative process after delivery. If one believes that HELLP syndrome develops as a sequela to a vascualr endothelium disruption process with prior oxidative damage to the endothelium and so forth, the recovery process would be expected to take several days.

Also, I would point out that 24% of the pregnancies in this study population were patients with recurrent HELLP. This includes many of the multiparous patients in this study.

Dr. Entman raised several excellent questions. He asked about the rationale that was utilized to develop the proposed classification scheme for HELLP syndrome. As we analyzed these data and treated these patients, it became very clear to us that there is a spectrum of disease for which there are no clear and absolute breaks in the data and thus no distinct groups. As I have lived and worked with these data for the last year or two, I have become convinced that the real break occurs at a platelet count of 50,000/mm^3. As the patient's platelet count falls below that amount, the amount of maternal morbidity increases substantially with a greater need for transfusions and more renal and pulmonary problems. Data regarding this were presented at the Tenth Annual Meeting of the Society of Perinatal Obstetricians, Houston, Texas, Janaury 23-27, 1990.

Did we identify all affected patients? I suspect we may have missed a few. The vicissitudes of medical record keeping and data collection preclude complete assurance that we identified every case. I think we studied just about every eligible parturient.

The ratio of aspartate aminotransferase to alanine aminotransferase was an incidental observation made in the concluding phases of data analysis. I do not know its significance.

Yes, we are looking currently at lactate dehydrogenase isozymes because the sixth lactate dehydrogenase isozyme is reportedly elevated in patients with particularly severe multisystem disease; at present I have no data to share.

A question was asked about neonatal morbidity and perinatal consideration. In a poster presentation at the Tenth Annual Meeting of the Society of Perinatal Obstetricians, our group reported that perinatal and neonatal problems increase in proportion to a worsening degree of HELLP syndrome. This is observed in relation to decreasing birth weight, lower Apgar scores, higher incidence of intraventricular hemorrhage, and so on. We did not find a consistent relationship with neonatal platelet counts.

Dr. Stedman and Dr. Perkins both raised the issue of whether mode of delivery could have an impact on recovery from disease. We have not analyzed our data on the basis of different modes of delivery. In this series 55% of our patients underwent cesarean delivery. Mode of delivery does not appear to have made a significant difference on disease pattern or recovery rate but we will go back and answer that question at a later time.

Dr. Coulam asked about the number of multiparous patients in this investigation. A total of 42% of the 302 patients, a larger percentage than we would have predicted, were multiparous. We reported at the Thirty-seventh Annual Meeting of the Soceity for Gynecologic Investigation, St. Louis, Missouri, March 21-24, 1990, that there were 94 subsequent gestations after class 1, 2, or 3 HELLP syndrome. Among these 94 there were 23 patients who had recurrence of HELLP syndrome, for a 24% recurrence rate. We have no data regarding the identity of sexual partners, so no conclusions can be derived regarding the same father versus a different father in a subsequent pregnancy.

Finally, Dr. Tomich asked about platelet transfusions, another excellent question. Our patients with class 1 HELLP syndrome received platelet transfusions in approximately 30% of cases. When we began the study, we were utilizing the practice of platelet transfusion when the maternal platelet count fell below 50,000/mm^3. We now know, and will report later this year in another presentation, that that cutoff point is probably too high because in our experience clinically significant bleeding does not occur until the platelet count decreases below 30,000/mm³. Therefore this value has become our cutoff point below which we protect patients with platelet transfusion whether they are to be delivered vaginally or abdominally. The overall incidence of transfusion is 60% in class 1 HELLP and 40% in class 2; virtually no patients in class 3 (platelet nadir >100,000/mm³ to ≤150,000/mm³) receive blood products.

We believe that the class 3 group requires further study. For practicing clinicians I think the class 3 grouping functions as a "bright yellow flag," prodding obstetricians to notice that patients are about to get into trouble as they fall into the class 2 and then class 1 grouping.

Suture entrapment and secondary ureteral obstruction

C. Robert Stanhope, MD, Timothy O. Wilson, MD, William J. Utz, MD,
Lynwood H. Smith, MD, and Peter C. O'Brien, PhD
Rochester, Minnesota

During the 6-year period ending in 1988, suture entrapment and secondary postoperative ureteral obstruction occurred in 18 (0.33%) of the 5379 patients who underwent major pelvic operations for benign conditions. Sixteen cases occurred after vaginal surgery and two after abdominal hysterectomy. Placement of the McCall suture or sutures for elevation of a bladder neck caused ureteral entrapment most frequently. Early diagnosis was facilitated by comparison of preoperative and postoperative serum creatinine levels. The mean change in serum creatinine level in patients with unilateral obstruction was an increase of 0.8 mg/dl. Treatment by either antegrade placement of ureteral stents or abdominal exploration with deligation or ureteroneocystotomy was successful in all cases. Retrograde placement of ureteral stents was unsuccessful. (AM J OBSTET GYNECOL 1991;164:1513-9.)

Key words: Postoperative ureteral obstruction, McCall's suture, high serum creatinine, percutaneous nephrostomy, antegrade ureteral stenting

Ureteral injury occurs infrequently after pelvic surgery for benign disease; however, the risk can be minimized by understanding the relationship of the ureter to other pelvic organs and, whenever possible, by identifying the ureter at operation. Identification and exposure of the ureter is readily accomplished during abdominal surgery because the retroperitoneal space can be entered easily and safely and the ureter can be identified throughout its course. This is not possible during vaginal surgery, especially if the course of the ureter is distorted by marked pelvic relaxation or previous pelvic surgery. We have reviewed our recent experience with ureteral obstruction after pelvic surgery for benign conditions to determine the frequency with which this occurs, to identify means for early diagnosis, and to describe our current management.

Patients and methods

In the 6-year period ending in 1988, 5379 major pelvic operations were performed for therapy of benign conditions at the Mayo Clinic. Of this group 2546 were vaginal hysterectomies or operations for correction of vaginal prolapse and 2833 were abdominal hysterectomies or other gynecologic abdominal operations for therapy of pelvic conditions. Eighteen cases of suture entrapment and secondary postoperative ureteral obstruction (15 unilateral and 3 bilateral) were found for an overall incidence of 0.33%. The obstructions were attributable to entrapment by ureteral ligation or ureteral kinking by the placement of a periureteral ligature. Ureteral perforation by a needle was not identified in any case. Sixteen cases occurred after vaginal operations, including repair of the vagina (incidence, 0.63%), and two occurred after pelvic operations done abdominally (incidence, 0.07%) (Table I). The site of

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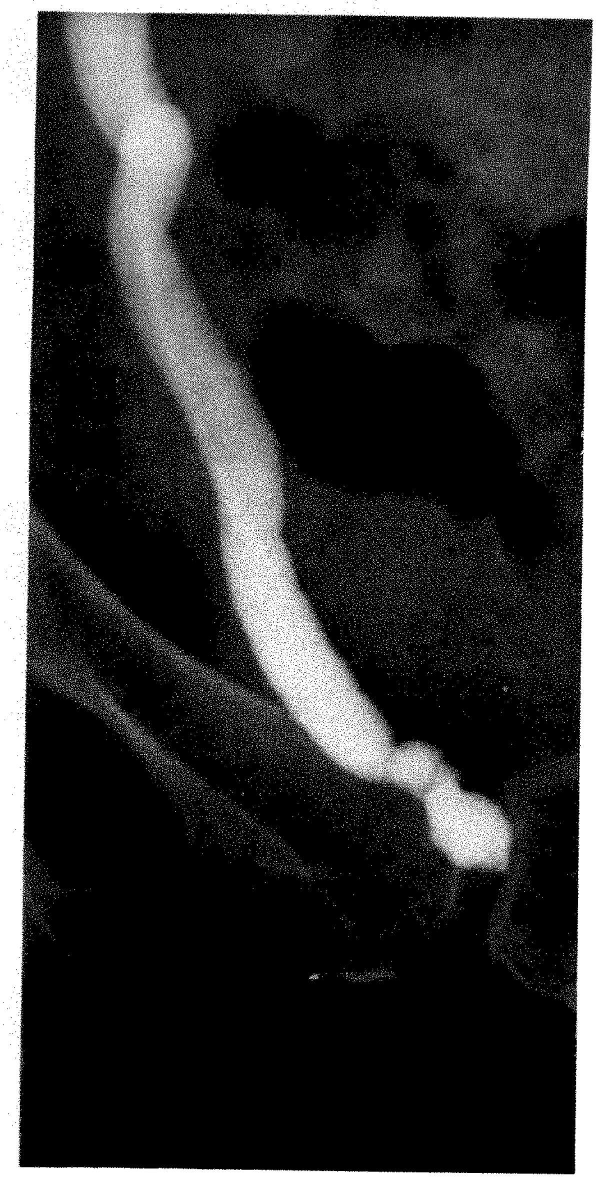

Fig. 1. Excretory urogram demonstrating ureteral obstruction after suture entrapment.

Table I. Distribution of postoperative ureteral obstructions

Original surgical procedure	*Obstruction (No.)*
Vaginal hysterectomy and repair for total procidentia	7
Vaginal hysterectomy and repair for grade 2 or 3 uterine prolapse or stress urinary incontinence	6
Vaginal repair of posthysterectomy vaginal vault prolapse	3
Total abdominal hysterectomy	2
TOTAL	18

Table II. Sites of ureteral obstruction

Site	*No.*
Unilateral	15
Left	9
Right	6
Bilateral	3

obstruction was the distal ureter in each case; the left side was involved more often than the right (Table II).

Flank pain, fever, or both occurred in 13 patients (72%) in the immediate postoperative period, and difficult micturition was noted in two patients. No patient experienced hematuria. Bilateral involvement was promptly recognized by anuria in three patients.

Signs of unilateral ureteral obstruction within the first 36 to 48 hours included an early transient increase in serum creatinine value in 17 of the 18 patients; one patient did not have a postoperative serum creatinine determination within that interval. In the evaluable patients with unilateral obstruction, the mean serum creatinine value was 0.93 mg/dl before operation and 1.65 mg/dl at 36 to 48 hours after operation. The mean change in serum creatinine level in patients with unilateral obstruction was an increase of 0.8 mg/dl (range, 0.3 to 1.4 mg/dl). Excretory urography, percutaneous nephrostography, or renal pelvic ultrasonography was used to confirm partial or complete unilateral ureteral obstruction (Figs. 1 and 2).

Three age- and procedure-matched controls for each of 13 cases of unilateral ureteral entrapment were identified. Serum creatinine levels increased markedly more in the cases than in the controls ($p < 0.001$; two-tailed paired *t* test) (Fig. 3). In a number of instances in the control cases, the postoperative creatinine value decreased slightly, presumably related to operative and immediate postoperative hydration.

Etiology. Although the ureter can be palpated during vaginal surgery and excluded from the vascular pedicles, it easily can become entrapped by the suture used to ligate a pedicle, to repair descent of the bladder neck, or to plicate the uterosacral ligaments.

Plication sutures at the base of the bladder approximate the pubovesicocervical fascia and correct the bladder neck descent while restoring the posterior urethral vesicle angle. It is our practice to place at least one McCall's suture to plicate the uterosacral ligaments and to obliterate any potential enterocele space in every patient undergoing vaginal hysterectomy or repair of vault prolapse. Often, two or more McCall's sutures are placed when there is significant enterocele or marked vaginal vault prolapse.

At least one McCall's suture was used in every vaginal case; however, two were used in five cases and three sutures were used in three cases. Either the McCall

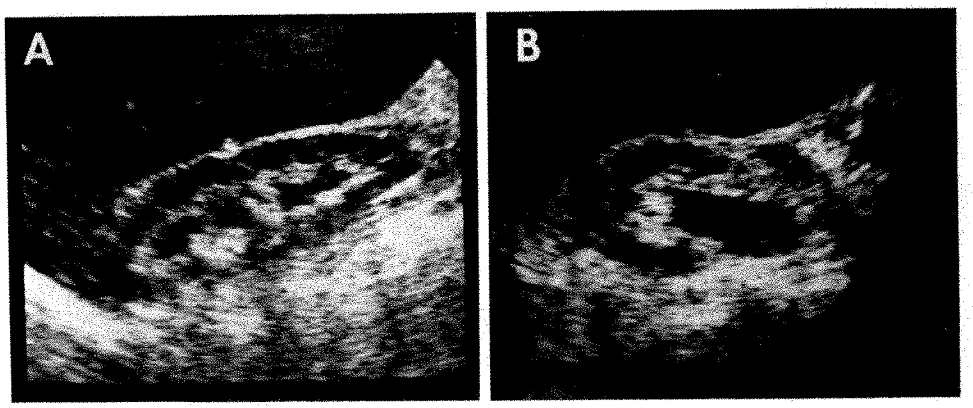

Fig. 2. Ultrasonograms of renal pelvis **(A)** and ureter **(B)**, demonstrating dilation of renal pelvis and ureter in association with ureteral obstruction caused by suture entrapment.

suture or the sutures used to repair bladder neck descent caused partial or complete obstruction in 14 cases. In one vaginal case a suture on a uterine artery pedicle caused entrapment, and in one vaginal case an infundibulopelvic ligament tie caused entrapment.

After abdominal hysterectomy a Moschcowitz uterosacral plication suture caused ureteral entrapment in one patient and a pelvic peritonealization suture caused entrapment at the pelvic brim in the second (Table III).

Rarely was the ureter actually encircled by a suture. Most commonly, the ureter was merely kinked by suture placement in the periureteral tissues.

Results

Attempted retrograde passage of a ureteral catheter at cystoscopy in 10 cases was unsuccessful. Retrograde ureteroscopy was attempted in one case but was not successful.

Percutaneous nephrostomy was performed in eight cases to achieve drainage of the affected renal unit, as well as to attempt antegrade passage of a ureteral catheter; subsequent stenting in this fashion was successful in six cases. Ten patients underwent early reoperation by the transperitoneal approach to mobilize the involved ureter(s) and, when indicated, to place ureteral stents through a cystotomy incision. Two patients required ureteroneocystostomy because the ureter was damaged or its vascular supply was compromised. Segmental ureterectomy and ureteroureterostomy were not indicated. No patient had a subsequent fistula, and all patients subsequently had normal renal function as measured by serum creatinine level. Subsequent excretory urography, performed in only a small group of patients, showed postoperative changes with no significant abnormalities.

Comment

The early recognition of ureteral obstruction is important because the degree to which nephron function is lost irreversibly is directly related to the duration and completeness of the obstruction. There are four areas within the pelvis where suture entrapment is most likely to occur: (1) the point where the ureter crosses the iliac vessels, (2) the point where the ureter comes into close proximity to the adnexa near the pelvic brim, (3) the point where the ureter is crossed by the uterine vessels, and (4) the base of the bladder where traction on the interureteric ligament may obstruct the ureter.

The relationship of the ureter to other pelvic structures during vaginal surgery has been examined during operating conditions.[1] The ureter is a mean of 1.0 cm from the clamp placed on the infundibulopelvic ligament when the tube and ovary are removed and a mean of 0.9 cm from the plication sutures used to provide support to the base of the bladder. However, despite this close proximity, ligation of the ureter or kinking is an uncommon occurrence. However, when it happens, it rarely is recognized during the operative procedure.

The frequency of postoperative ureteral obstruction reported in the literature ranges from 0.1% to 2.4%.[2-4] Most of the reported cases involve injury occurring during abdominal hysterectomy for benign conditions. The present study differs in that only rarely (2/18) did the injury occur during abdominal hysterectomy, and the vaginal surgery associated with injury involved uterine or vaginal prolapse in every case.

Early recognition of ureteral obstruction in the postoperative period, even before flank pain or fever develops, is possible by detecting subtle increases in the serum creatinine value, which can begin as early as 24 hours after operation. The serum creatinine value may return to nearly normal by 72 hours after operation. This phenomenon has been observed under experimental conditions in the case of unilateral ureteral obstruction.[5]

With total unilateral obstruction, initially there will be a transient increase in renal blood flow, lasting 1 to 2 hours. This will be followed by vaosconstriction and

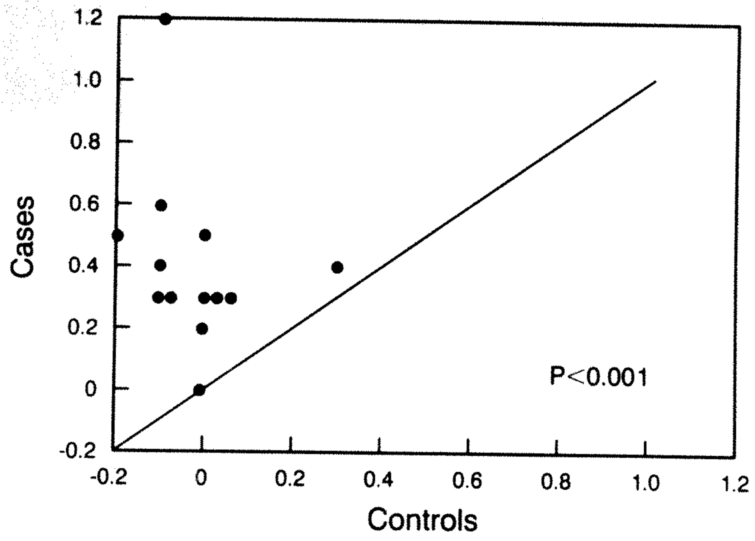

Fig. 3. Increases in serum creatinine level (milligrams per deciliter) were greater in 13 patients with unilateral ureteral obstruction *(Cases)* compared with mean of three age- and procedure-matched patients who had no obstruction *(Controls)* ($p < 0.001$).

Table III. Cause of ureteral obstruction*

Cause	*No.*
McCall's suture or bladder neck suspension	8
Uterovascular pedicle	1
Ovarian vascular pedicle	1
Moschcowitz uterosacral plication	1
Pelvic reperitonealization	1

*Six cases were treated successfully with antegrade placement of a ureteral stent, so cause was not determined.

a progressive decrease in renal blood flow with a decrease in glomerular filtration rate, which is marked by 24 hours. The situation is similar to that after nephrectomy—an initial increase in creatinine value and a slow decrease back to normal in a matter of weeks, as the contralateral kidney hypertrophies functionally.[6]

Historically, the first attempt at treatment was cystoscopy, retrograde pyelography, and attempted passage of a ureteral catheter or stent. Although retrograde pyelography has been useful to document the point of obstruction, this information is of little added clinical benefit. All 10 attempts to pass a ureteral stent (after retrograde pyelography) past the point of obstruction failed.

Ureteroscopy is a valuable new technique for the diagnosis and treatment of some cases of ureteral obstruction.[7] In some situations in which a catheter cannot be placed cystoscopically, endoscopic inspection usually can identify the site of obstruction through which a wire or stent can be passed. This procedure was attempted in one patient but was not successful. Additional experience with this procedure may be useful.

Early percutaneous nephrostomy is advisable to provide drainage of the renal unit and to preserve renal function. This obviously is significant with bilateral obstruction, but it is also important with unilateral obstruction. An antegrade ureteral stent was passed successfully 24 to 48 hours later in six of eight attempts in cases of unilateral obstruction. When antegrade ureteral stent placement was not successful, the exact nature of the entrapment was determined and the ureter(s) was released surgically.

The issue of immediate reoperation in the early postoperative period or delayed operation remains controversial. After percutaneous nephrostomy, a "wait-and-see" approach without stent placement has been advocated[8] when ureteral obstruction is secondary to entrapment by an absorbable suture. On the basis of our experience, if antegrade stent placement is not possible, early exploration has been well tolerated and well accepted by our patients without occurrence of ureteral fistulas or loss of renal function.

In conclusion, the nature of pelvic surgery and the many anatomic variations encountered create a setting in which suture entrapment and secondary ureteral obstruction can occur, regardless of the experience of the surgeon. Four conclusions can be drawn from our experience.

1. Small increases in serum creatinine level 36 to 48 hours after pelvic surgery suggest postoperative unilateral ureteral entrapment.

2. Suspected abnormalities should be confirmed with either excretory urography, percutaneous nephrostography, or ultrasonography of the renal pelvis and ureter; the latter is an extremely sensitive, noninvasive pro-

cedure that is not dependent on renal function or dynamic renal scanning. Retrograde pyelography and attempts at retrograde placement of ureteral stents are not successful; however, ureteroscopy may make it possible to place a stent proximal to the obstruction.

3. Percutaneous nephrostomy to drain the kidney should be performed early, and antegrade pyelography with placement of a ureteral stent is desirable.

4. Abdominal exploration with ureteral mobilization is needed when a stent cannot be passed beyond the point of obstruction or the kidney cannot be drained adequately. Removal of the offending suture(s) or ureteroneocystostomy is indicated to avoid loss of renal function or further problems, such as fistula formation.

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Discussion

DR. BRUCE H. DRUKKER, East Lansing, Michigan. Once again our colleagues from the Section of Gynecologic Surgery at the Mayo Clinic have tapped their substantial surgical experience and provided us with interesting material for comment, speculation, and inquiry.

The fact that approximately 900 pelvic procedures per year for benign disease were done during the 6-year study period exemplifies the magnitude of the resource, and the low occurrence of entrapment (0.33%) focuses on the excellence of technique of these high-volume gynecologic surgeons.

Ureteral entrapment is a concern for all gynecologic surgeons. This article focuses on the interesting observations of renal physiologic change, which are useful for diagnosis, and on the fact that almost all of the entrapment situations occurred during vaginal surgery. Further, it appears that the so-called McCall suture, singly or in multiples, or suture to repair bladder neck descent, was the offending suture in at least eight of 18, or 44%, of cases. One can speculate that some other entrapments were also due to these sutures; however, that diagnosis could not be confirmed because of the passage of a percutaneous ureteral stent. Could the authors tell us which suture sequence, the McCall or that used for bladder neck descent correction, caused entrapment more frequently? Although entrapment occurred infrequently in these cases, it did occur in 0.63% of vaginal cases compared with only 0.07% of abdominal cases. Why is this? Do Dr. Stanhope and his colleagues routinely expose the ureter in all pelvic procedures for benign conditions? If they do, what does that really mean—"to expose"? Is the peritoneum lateral to the ovarian vessels opened? Is the broad ligament opened to reveal the ureter? Is the dissection carried lower into the so-called cardinal ligament area? The reason I ask is because "to expose" means different things to different people. With respect to the vaginal cases, ureteral exposure, of course, is not routine and the ureter generally is not noted. However, the adage of "staying close to the uterus" seems to save most of us from damaging the ureter. On the other hand, particularly in vaginal hysterectomy for grade II or grade III prolapse, there are a few gynecologic surgeons who say you can routinely palpate the ureter. Most of us probably are somewhere in between. If there is a potential problem with the McCall suture and its use continues, as I suspect it does, what precautions are taken to avoid this problem? Has the suture been placed differently or are different clamps used? Certainly, the Haney clamp does take a stout amount of pedicle tissue.

But let's go on to some more valuable material. The authors cite an interesting reference evaluating the relationship between renal blood flow and ureteral pressure during an 18-hour period of total unilateral ureteral occlusion. This citation of basic science is particularly intriguing because it appears to be the clue the authors followed that suggests that transient 24- to 72-hour increases in serum creatinine do, in fact, occur after unilateral ureteral entrapment. This observation can lead to early suspicion of entrapment, and, as recommended by the authors, prompt, early treatment may salvage some renal glomerular units. Serum creatinine is a fairly standard preoperative assessment, but what triggered Dr. Stanhope and his colleagues to obtain postoperative creatinine assessments? Was it fever or flank pain? Or do they routinely order this study in all patients undergoing benign gynecologic surgery? That thesis is unlikely but possible. Since fever can occur from other sources in the immediate postoperative period, it seems flank pain would have been the discriminating system. I am sure the authors will shed some light on this. I would also like to know how they monitor recovery of damaged renal units and how one can determine return to normal function. In this situation creatinine seemingly is not helpful because the opposite kidney takes over function quite quickly. Did they evaluate creatinine clearances in these patients?

Now, to the next observation. Retrograde cystoscopically guided ureteral catheter placement does not seem to be very effective treatment for this problem, having failed in 10 attempted cases. On the other hand, six of eight patients underwent successful percutaneous nephrostomy with ureteral catheter placement. It appears that this is the way to go in the future. Have the authors now abandoned attempted retrograde ureteral catheter placement on the basis of their past experience, or do they feel it should at least be given a try? If it is effective, do they feel that a double J stent placed in a retrograde fashion does a better job than the percutaneous procedures? Also, when percutaneous catheters were inserted, what indicator was used relative to the time of removal? Were the indicators for removal the same as for those stents placed at the time of abdominal reoperation when the stent was placed by open cystotomy?

Dr. Stanhope and his colleagues have given us some interesting material to consider, but by this article they call to our attention that care should be taken to understand the anatomic characteristics of the ureter, particularly in patients with severe uterine or vaginal prolapse. Basic gynecologic surgery should not be taken for granted. It is an art that can be learned and practiced but periodically should be reassessed.

Congratulations to Dr. Stanhope and his colleagues for giving us some important information relative to serum creatinine and ureteral anatomy. It is a contribution that will help all of us.

Dr. Robert E. Rogers, Indianapolis, Indiana. When I review an article concerned with a surgical complication, I generally ask two questions: (1) How may I avoid the complication in my practice? (2) If I am faced with the complication, how is it diagnosed and treated? The answer to the first question is not in the purview of this study, but there are answers here.

Ureteral injury is avoided by ensuring that the operative site is fully exposed, adjacent organs are localized, and hemostasis is established by clamping and suturing structures that are identifiable and visible. In the study group at abdominal operation, when the surgeon was able to expose and identify the ureter, only two ureteral injuries were found in 2833 operations. One injury was related to pelvic peritonealization and another to a Moschcowitz cul-de-sac obliteration. One might speculate that these might have been avoided if the pelvic peritoneum had not been closed and if the cul-de-sac had been closed with a Halban[1] procedure. Nevertheless, this is an excellent record.

Sixteen injuries incurred in the course of 2546 vaginal operations should serve as a warning to those of us who treat increasing numbers of women with genital prolapse. Genital prolapse distorts pelvic anatomic relationships and places the ureter in harm's way. The solutions to this continuing problem await further study, but the authors have reminded us of the danger of the McCall culdoplasty. Their findings confirm the fact that when the ureter cannot be fully identified and exposed and pedicles isolated, injury will occur.

The low injury rate in abdominal operation is a testament to the ability of this group of surgeons to identify and isolate the ureter. Most reports[2] of ureteral injury state that three fourths of all ureteral injuries result from abdominal surgery whereas one fourth result from vaginal surgery. In this series the predominance of injury at vaginal operation is undoubtedly related to the demand for the care of complex genital prolapse.

The authors describe a laboratory test that points to a diagnosis of operative ureteral injury. The study is well conceived and well controlled, and the results are conclusive. I was surprised at the result. I have long believed that renal reserve was so great that the loss of function of one kidney in the presence of a contralateral normal kidney was of little physiologic consequence, even in the short term. The studies of Moody et al.[3] in dogs have shown that ureteral obstruction is followed by a transient increase in renal blood flow, followed by vasoconstriction and then a decrease in renal blood flow. In the early days after ureteral injury the increased requirement for filtration cannot be immediately met by the healthy kidney, and the result is a transient increase in creatinine. A transient rise in serum creatinine levels within 36 to 48 hours after operation is highly suggestive of ureteral obstruction. Thus the authors have taken an interesting laboratory observation and turned it into what may be a useful clinical tool. When these warnings are heeded, it will allow the surgeon to evaluate the urinary tract for injury early enough to save a kidney.

I am convinced that ureteral obstruction elevates creatinine levels postoperatively after 36 and 48 hours. My only concern is for the utility of the information. The problem is that many of us do not measure serum creatinine levels preoperatively, and if we did, measurements most likely would not be repeated postoperatively unless the patient was having postoperative problems.

It appears that the study group consists of patients with established ureteral injury who were studied retrospectively in regard to creatinine status. Did the authors review the results of creatinine studies in asymptomatic patients, seeking patients with asymptomatic (silent) ureteral injury, which in some studies account for >50% of the injuries?

Does the author feel that the test he describes is sufficiently useful that paired creatinine levels should be collected on all pelvic surgical patients?

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Dr. Pedro A. Poma, Melrose Park, Illinois. I have two questions. First, did the authors review all the other cases of elevated creatinine, follow them, and find out how many *had* ureteral obstruction? Second, what is the influence of dehydration, blood loss, and fever in relationship to the postoperative creatinine levels?

Dr. Daniel Rightmire, Springfield, Illinois. I wanted to ask how often do the authors believe that unilateral entrapment of the ureter goes undetected, because that's certainly important in this case. Second, may I suggest that the change in serum creatinine data be submitted to receiver operating characteristic curve analysis?

Dr. Stanhope (Closing). I would like to thank Drs. Drukker and Rogers and Drs. Poma and Rightmire from Illinois for their comments.

Concerning Dr. Drukker's first concern, the probable and more likely site for ureteral entrapment is compression of the interureteric ligament. The McCall suture also has been documented to cause entrapment, and I have taken photographs of this. But I believe the greatest risk is traction on the interureteric ligaments, causing the ureter to kink.

The McCall suture, as described in 1957 and published in the *Green Journal,* consisted of three silk sutures designed to approximate the uterosacral ligaments from their posterior and lateral perirectal positions. If you draw an imaginary transverse line, bisecting the surgical field, and place the sutures well below that line, you can be reasonably assured entrapment will not occur.

Dr. Drukker's second concern was how we expose the ureter during abdominal operations where the ovaries or just the uterus is removed. It is our practice to open the broad ligament and identify visually the course of the ureter down to where the uterine artery crosses it. Then, when a clamp is placed on the uterus at the level of the uterine artery, we palpate the ureter. We do this in every case.

This report just dealt with surgery for benign conditions. However, our frequency of ureteral entrapment or obstruction after surgery for malignant conditions is less, related to the fact that most surgery for cancer is abdominal and the ureter is often completely dissected in cancer operations.

Finally, about retrograde ureteral studies, we don't attempt retrograde stent placement at all anymore. But there is a new procedure, called ureteroscopy. With a fiberoptic instrument the urologist can get into the ureter, advance a guide wire that can turn and maneuver around an apparent kink, and position a ureteral stent. Ureteroscopy may play a future role.

Dr. Rogers had expressed concern about obtaining serum creatinine levels. Serum creatinine levels were obtained preoperatively in all of our patients as part of our preanesthetic medical evaluation. We get postoperative electrolyte studies that also include serum creatinine. So, for a number of years, we have had this information available for both the preoperative and the postoperative periods.

I believe that this test is significant, with a high observed sensitivity of 100% and an observed specificity of 92%, and that we should obtain paired serum creatinine levels preoperatively and postoperatively, as you indicated, in patients undergoing hysterectomy or surgery where ureteral injury could occur. I think we should follow through on this.

Dehydration can cause an increase in the serum creatinine level. Serum creatinine may be elevated in the preoperative period when the patient has fasted, and then postoperatively, after hydration, it may be lower than the preoperative value.

Obviously, there are other causes for elevated creatinine, for example, the use of aminoglycosides.

I think that in our series there are probably undetected incidences where ureteral entrapment has occurred. We were not trying to look at that. We got the idea for this study from a nephrologist who indicated that this phenomenon occurred in patients who had unilateral ureteral stones and that these patients were found to have transient rises in serum creatinine. I noted that we frequently saw a rise after ureteral entrapment from vaginal surgery, so we decided to look at this in more detail.

I will take your suggestion for further statistical analysis.

Stage IB squamous cell cancer of the cervix: Clinicopathologic features related to survival

Michael P. Hopkins, MD, and George W. Morley, MD
Ann Arbor, Michigan

Three hundred forty-five patients with stage IB squamous cell carcinoma of the cervix were treated at the University of Michigan Medical Center from 1970 through 1985. The overall cumulative 5-year survival was 89%. The clinical characteristics included mean age 44.6 years, nulliparity 10%, married 93%, obese 38%, hypertension 32%, diabetes mellitus 5%, smoking 54%, symptoms of bleeding 68%, positive cytologic smear 83%. Lymph nodes were diseased in 45 of 261 (17%) with 26 unilateral (10%) and 19 bilateral (7%). Tumor differentiation showed: grade 1, 112 (33%); grade 2, 144 (42%); grade 3, 86 (25%). Factors that did not influence survival included age, presence or absence of positive cervical cytologic smear, the interval from previous papanicolaou smear, hypertension, smoking history, patient's blood type, and transfusion at radical hysterectomy. In all patients survival was significantly influenced by the following features: tumor classified as well differentiated (95%) or poorly differentiated (82%); tumor size <3 cm (91%) or >3 cm (76%); negative lymph nodes (93%) or positive lymph nodes (61%). When three or fewer lymph nodes were involved, the survival was 79% compared with 33% when four or more lymph nodes were involved. In 213 patients undergoing radical hysterectomy the cumulative 5-year survival was significantly influenced by the amount of residual cervical disease: no residual disease, 100%; <50% penetration, 96%; >50% penetration, 83%. Involvement of the lower uterine segment reduced survival to 73% compared with 95% when the lower segment was uninvolved. One hundred seventeen patients without angiolymphatic invasion had a 97% cumulative 5-year survival whereas 70 patients without disease in the lymph nodes but with angiolymphatic invasion had an 88% cumulative 5-year survival rate. A Cox model, multiple proportional hazard analysis was performed for all patients, and the factors that influenced survival included tumor grade, tumor size, presence of metastatic disease in the lymph nodes, and diabetes mellitus. In patients undergoing radiation therapy, the tumor grade and size were significant factors in survival. In patients undergoing radical hysterectomy, survival was influenced by the depth of cervical penetration and lower uterine segment involvement whereas the tumor grade, tumor size, patient's age, and removal of ovaries were not significant. (AM J OBSTET GYNECOL 1991;164:1520-9.)

Key words: Cervix cancer, squamous cell cancer

The survival in early stage invasive squamous cell cancer of the cervix is influenced by a number of features.[1-9] These are important from a prognostic standpoint but oftentimes do not alter the standard therapy of either radiation or radical surgery. As effective adjuvant therapies become available, these prognostic factors will be critical to determine which patients would benefit from adjuvant therapy. This study was undertaken to evaluate the clinicopathologic features related to survival in patients with stage IB squamous cell cancer of the cervix.

Material and methods

The clinical records, pathology reports, and gynecologic tumor conference notes for patients at the University of Michigan Medical Center with a diagnosis of carcinoma of the cervix for the period 1970 through 1985 were reviewed. The information for the clinical presentation, medical history, treatment modality, and size of the cervical lesion were recorded. All patients had staging of cancer according to the International Federation of Gynecology and Obstetrics (FIGO) guidelines. Patients with squamous cell carcinoma diagnosed after a standard hysterectomy without obvious parametrial involvement were considered to have stage I disease. These patients ($n = 21$) were only considered in the overall survival analysis. Microinvasive squamous cell carcinoma (stage IA), as previously reported from our institution, was defined as ≤3 mm of invasion without angiolymphatic invasion.[10] All patients with invasion >3 mm or angiolymphatic invasion were considered to have stage IB disease.

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6/6/28917

The original hematoxylin and eosin slides were reviewed. When the original slides were unavailable, the original pathology report and the gynecologic tumor conference note were utilized. All patients with an adenocarcinoma or adenosquamous carcinoma were excluded. Well-differentiated tumors were defined as those neoplasms where there was little nuclear pleomorphism and the nuclear cytologic atypia was not pronounced. Cells were generally large with abundant cytoplasm and a more normal nuclear/cytoplasmic ratio. In general, the well-differentiated tumors produced keratin, and mitotic figures were infrequent. Poorly differentiated tumors had pronounced nuclear pleomorphism, hyperchromatic nuclei, and scant cytoplasm. Cells were smaller, mitoses were frequent, and keratin formation was usually absent. Moderately differentiated tumors were intermediate in characteristics.

Only the patients who underwent radical hysterectomy were included in the survival analysis for the features of depth of penetration, angiolymphatic invasion, and lower-segment involvement. All these features were not known with certainty in patients who underwent radiation. The depth of invasion was measured by the residual disease in the hysterectomy specimens. Approximately 12 longitudinal sections through the cervix and lower uterine segment had been made at the time of the initial surgery. Thus the cervix, endocervix, and lower segment could be evaluated. Light microscopy of the slide with the maximum depth was used to evaluate the amount of residual disease. When necessary, a micrometer was used to determine if the lesion invaded >50% into the cervix. The lower segment was considered to be involved when disease extended into areas of endometrial stroma or glands. The mean number of lymph nodes removed at the time of pelvic lymph node dissection was 24. Lymph nodes were routinely cut into three sections for review.

Statistical analysis was performed by the Department of Biostatistics, School of Public Health, University of Michigan. Survival was plotted with the life-table method described by Kaplan and Meier.[11] The Savage (Mantel-Cox) method was used to test differences in survival.[12,13] A Cox model, stepwise proportional hazard analysis was used to compare the hazard for tumor-related deaths related to potential risk factors.

Results

During the time period 1970 to 1985, 345 patients with stage IB disease were identified. The overall cumulative 5-year survival was 89%. There was no difference in survival between those treated with radical surgery and those who received radiation therapy. The clinical characteristics included a mean age of 44.6 years (range, 18 to 89 years). Parity was available on 334 patients; 35 (10%) were nulliparous, 53 (15%) had one child, 257 (75%) had more than one child. Three hundred twenty-two (93%) were married, 132 (38%) were considered to be obese as defined by 20% above ideal body weight for height, hypertension was present in 109 (32%), and diabetes was present in 18 (5%). A smoking history was available for 320 patients, and 174 (54%) smoked within 1 year of diagnosis. Bleeding as a presenting symptom was present in 232 (68%), and 248 (83%) had a positive cytologic smear leading to the diagnosis. A previous, normal cytologic smear had been obtained within ≤1 year in 145 (47%), whereas 82 (30%) had not had cytologic testing performed in >3 years. The previous normal cytologic smears were not available for review. Oral contraceptives were used by 55 of 148 patients (37%) under age 40. The lymph node status was available on 261 patients with stage IB disease: 216 (83%) were negative, 26 (10%) had unilateral positive nodes, and 19 (7%) had bilateral positive nodes. Tumor differentiation was available for 342 patients: 112 (33%) had grade 1 tumors, 144 (42%) had grade 2 tumors, and 86 (25%) had grade 3 tumors. Size was evaluated according to those patients who had lesions ≤3 cm in size versus those whose lesions were >3 cm. Two hundred eighty-three patients (82%) had tumor size ≤3 cm whereas 61 (18%) had a tumor size >3 cm.

Radical hysterectomy was performed on 25 patients with invasion between 3 and 5 mm and 5 patients with <3 mm of tumor invasion but with angiolymphatic invasion. None of these 30 patients had node metastases and none has had a recurrence.

A survival analysis was performed for all patients. Factors that did not influence survival included age, the presence or absence of positive cervical cytologic smear, the interval from previous Papanicolaou smear, presence of hypertension, a history of smoking, patient's blood type, and whether transfusion was done at the time of radical hysterectomy. When all patients with stage IB disease were evaluated, the factors that significantly influenced survival included tumor trade, size of lesion, node status, and presence of diabetes. Patients with well-differentiated tumors had a 95% survival compared with an 82% survival in those with poorly differentiated tumors (Fig. 1). Patients with tumor size <3 cm had a 91% cumulative 5-year survival compared with a 76% cumulative survival in those with >3 cm (Fig. 2). The lymph node status was available for 261 patients, 213 of whom underwent radical hysterectomy with pelvic node dissection. Fourteen patients underwent surgical exploration for radical hysterectomy but did not have the surgery completed. The remaining patients had lymphangiography with directed needle biopsy for evaluation of lymph nodes. Patients without diseased lymph nodes had a 93% cumulative survival,

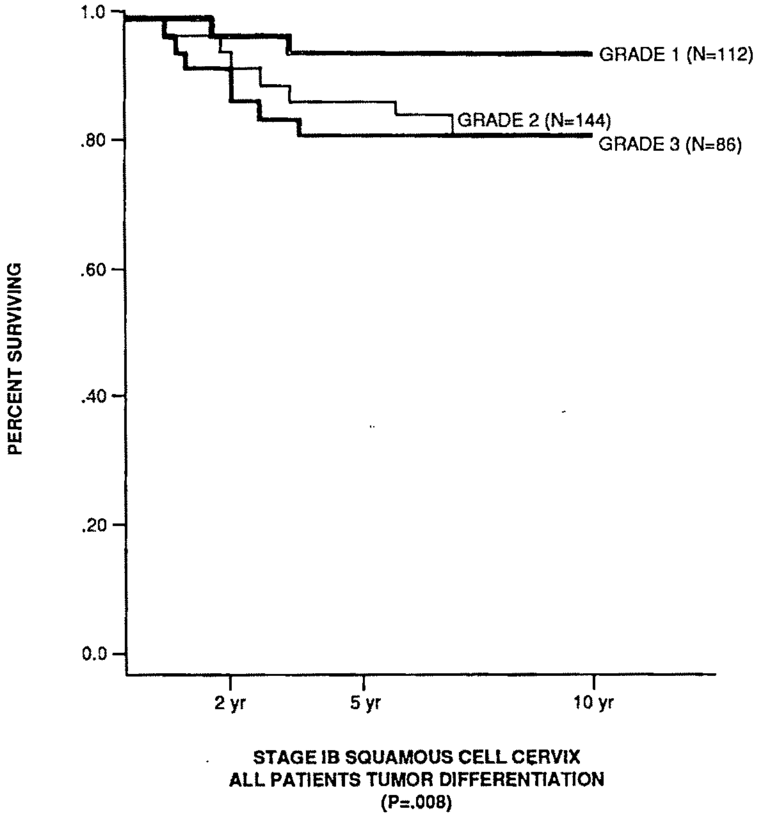

Fig. 1. Survival for all patients with stage IB disease according to tumor grade.

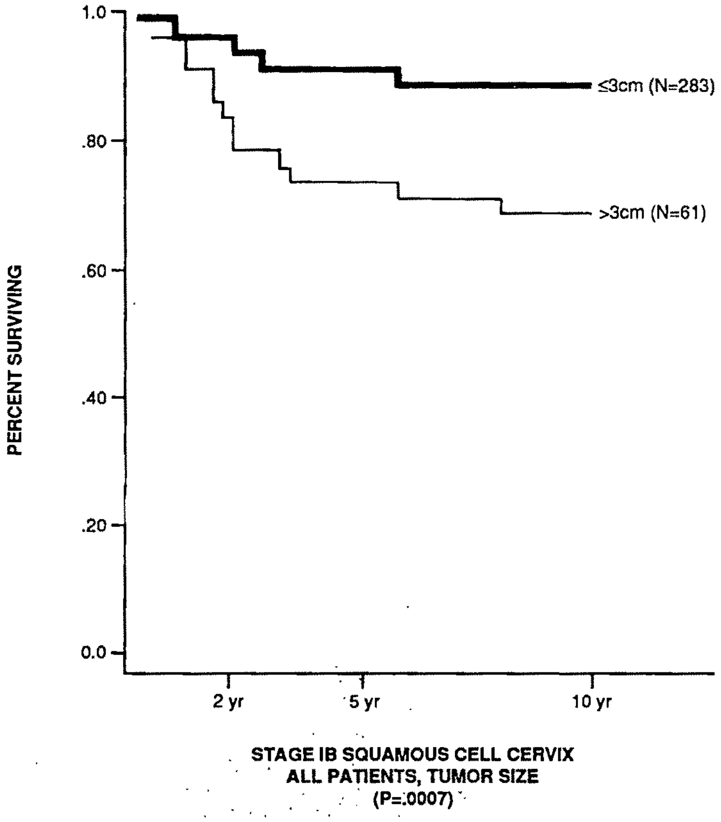

Fig. 2. Survival for all patients with stage IB disease according to tumor size.

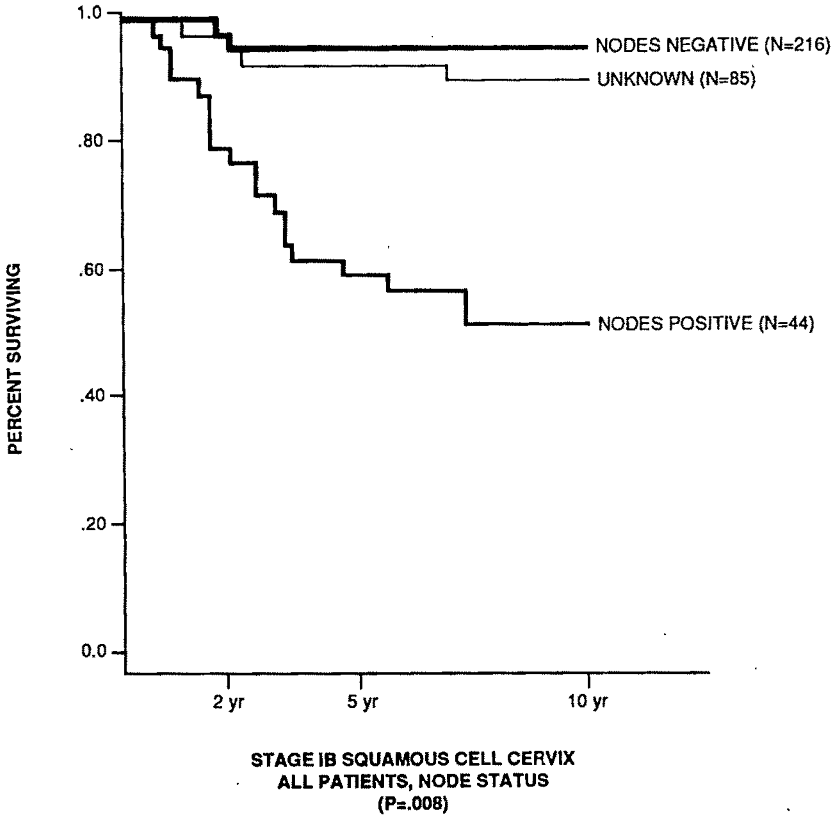

Fig. 3. Survival for all patients with stage IB disease according to lymph node status.

85 patients with an unknown lymph node status had a similar 5-year survival, and patients with lymph node metastases had a cumulative 5-year survival of 61% (Fig. 3). The patients with diseased lymph nodes were evaluated according to the number of lymph nodes involved. Patients with three or fewer involved lymph nodes had a 79% cumulative 5-year survival, whereas those with more than three involved lymph nodes had a 33% cumulative 5-year survival (Fig. 4). The presence of unilateral or bilateral lymph node metastases influenced survival. The patients with unilateral metastatic disease had a 75% cumulative survival, and those with bilateral metastases had a 42% cumulative survival. When all patients were evaluated, diabetes influenced survival. Eighteen patients with diabetes had an 82% cumulative 5-year survival compared with 327 patients without diabetes who had an 89% survival ($p = 0.04$). Patients with diabetes were generally older, were in poorer medical condition, and were more likely to undergo radiotherapy.

Patients who underwent radical hysterectomy were then evaluated. The depth of cervical penetration had a significant influence on survival. Additionally, the depth of penetration significantly influenced the chance of node metastases (Table I). Patients with no residual disease in the cervix at the time of radical hysterectomy had a 100% cumulative 5-year survival. Patients with <50% penetration had a 96% cumulative survival whereas those patients with ≥50% penetration had an 83% cumulative 5-year survival (Fig. 5.) Involvement of the lower uterine segment significantly influenced survival. When the lower segment was involved, survival was 73% compared with 95% when it was not involved (Fig. 6).

The significane of angiolymphatic invasion for survival was evaluated for those patients who underwent radical hysterectomy and had negative lymph nodes. One hundred seventeen patients without angiolymphatic invasion had a 97% cumulative 5-year survival whereas 70 patients without diseased lymph nodes but with angiolymphatic invasion had an 88% cumulative 5-year survival (Fig. 7). Radiation therapy was added in 10 patients with angiolymphatic invasion and this did not influence survival. An evaluation of survival according to the amount of residual tumor in the radical hysterectomy specimen was performed for the 70 patients with angiolymphatic invasion. Patients with angiolymphatic invasion but without residual disease in the cervix had a 100% cumulative 5-year survival. Patients with less than half of the cervix involved but with angiolymphatic invasion had a 94% cumulative 5-year survival whereas patients with angiolymphatic invasion and cervical penetration of ≥50% had a 78% cumulative 5-year survival (Fig. 8).

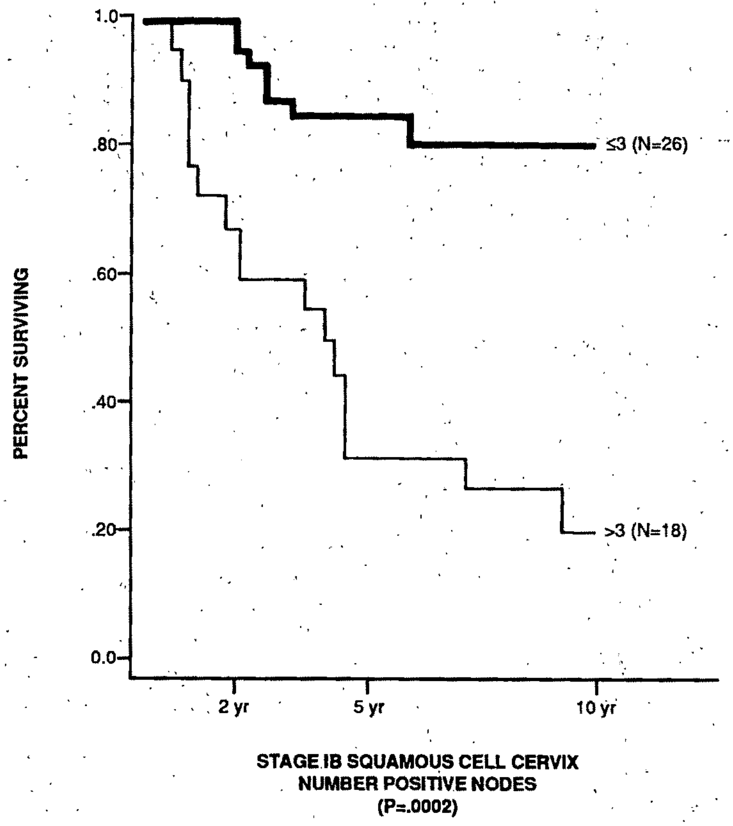

Fig. 4. Survival for all patients with stage IB disease according to number of lymph nodes with metastatic disease.

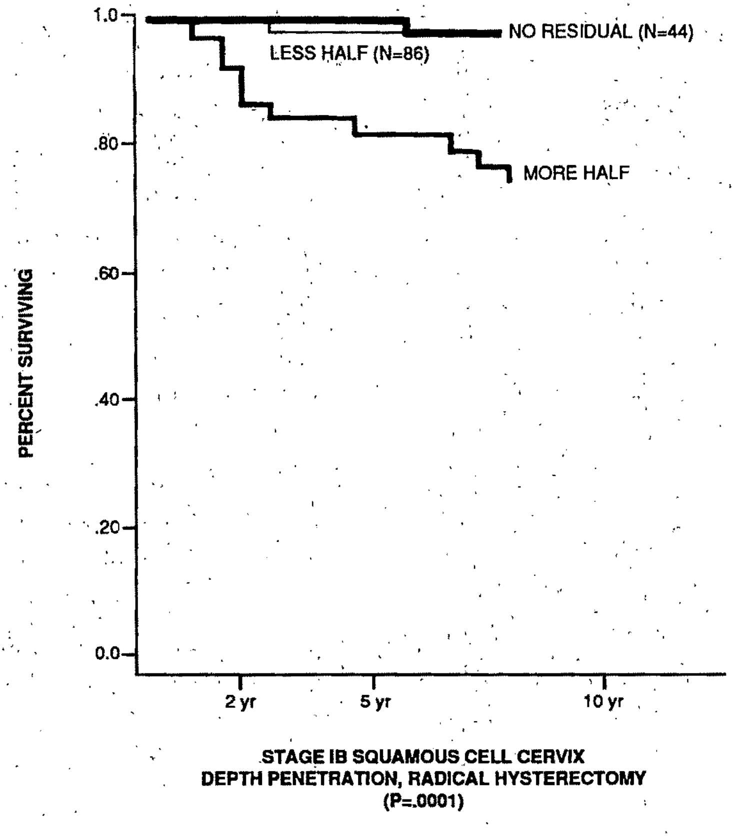

Fig. 5. Survival for patients undergoing radical hysterectomy according to depth of cervical penetration.

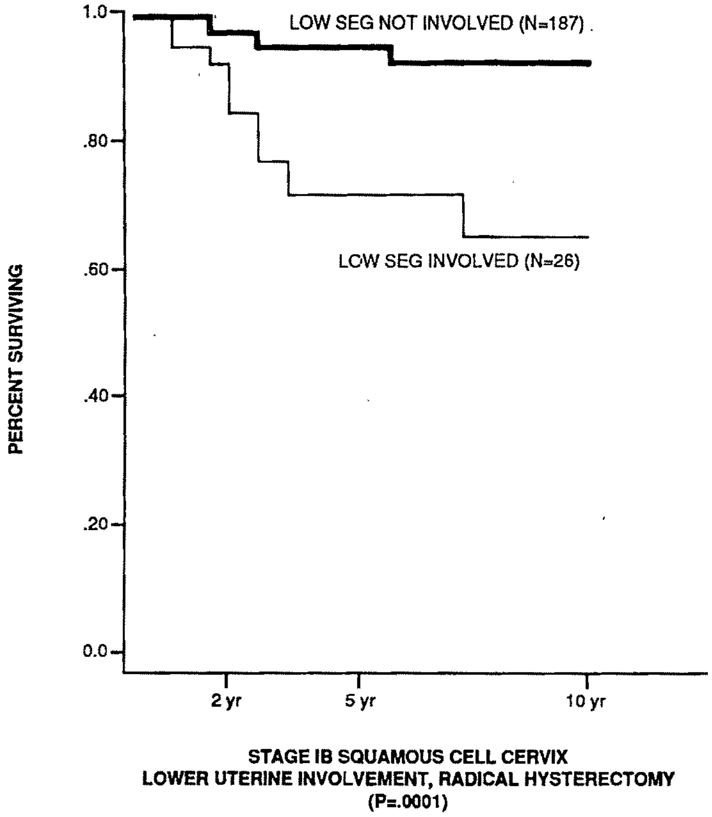

Fig. 6. Survival for patients undergoing radical hysterectomy according to presence of disease in lower uterine segment.

A Cox model, multiple proportional hazard analysis was performed for various subsets. When all patients were analyzed, the factors found to significantly influence survival included the tumor grade, size of the cervical lesion, presence of metastatic disease to the lymph nodes, and diabetes. Factors that did not influence survival included the patient's age, the interval since the previous cytologic smear, the finding of a positive cytologic smear, or a history of smoking. A Cox model analysis was performed only for those patients who underwent radical hysterectomy. In these 213 patients the factors found to significantly influence survival included a depth of cervical penetration of ≥50% ($p = 0.0001$); lower-segment involvement approached significance ($p = 0.06$). The tumor grade, tumor size, and age of the patient were not significant in the Cox model. The lymph node status was not significant in the Cox model, and this was due to the greater number of patients who had positive nodes when the depth of cervical penetration was considered. A Cox model analysis was performed for patients who underwent radical hysterectomy and had negative lymph nodes. In this model the depth of cervical penetration ($p = 0.001$) and the presence of angiolymphatic invasion ($p = 0.001$) significantly influenced survival whereas grade, size, age, and lower-segment involvement did not. In a Cox model analysis performed for patients undergoing radiation therapy the tumor grade and tumor size were significant for survival.

Table I. Node status according to amount of residual disease in cervix at radical hysterectomy ($p < 0.0001$)

Cervix	*Negative nodes*	*Positive nodes*	
		≤3	*≥4*
No residual	43	1	0
<50% Penetration	84	2	0
≥50% Penetration	60	16	7

Comment

The effect of tumor differentiation in squamous cell cancer has been evaluated with variable results. In our previous experience, as well as in this report, patients with a poorly differentiated histologic type had a decreased survival. In the previous time period, 87% with a poorly differentiated lesion survived to 5 years while 82% survived during the current time period.[14] Creasman et al.[3] found no difference in survival on the basis of tumor grade. Gauthier et al.[6] did not find the grade to be significant in 100 radical hysterectomy specimens evaluated. Underwood et al. found that survival dropped to 74% for patients with poorly differentiated

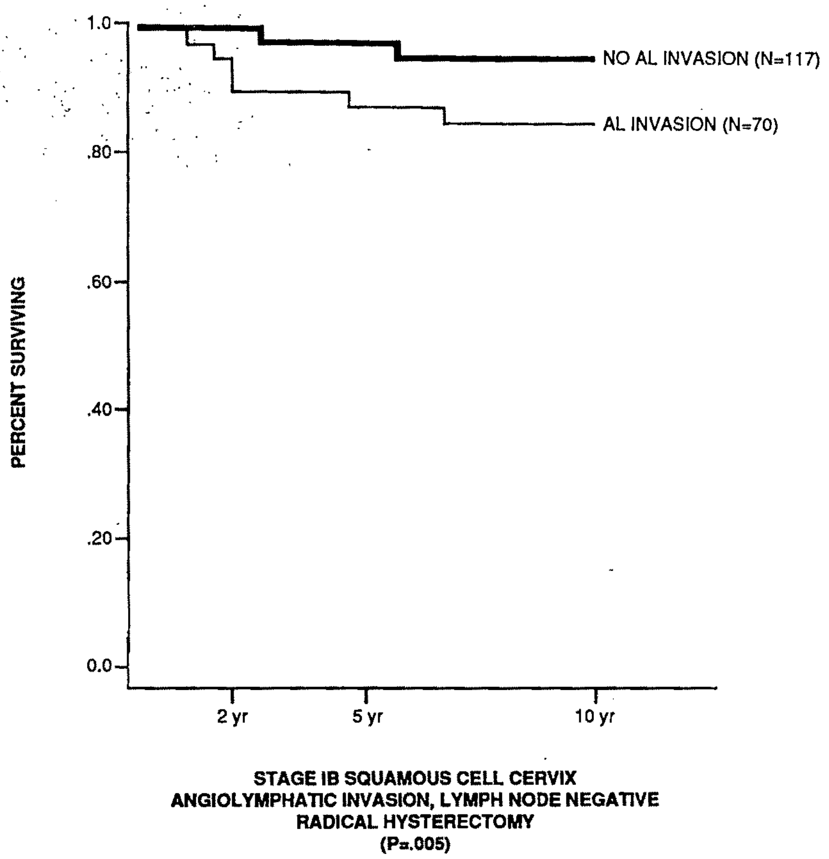

Fig. 7. Survival for patients undergoing radical hysterectomy with and without angiolymphatic *(AL)* invasion, with negative lymph nodes.

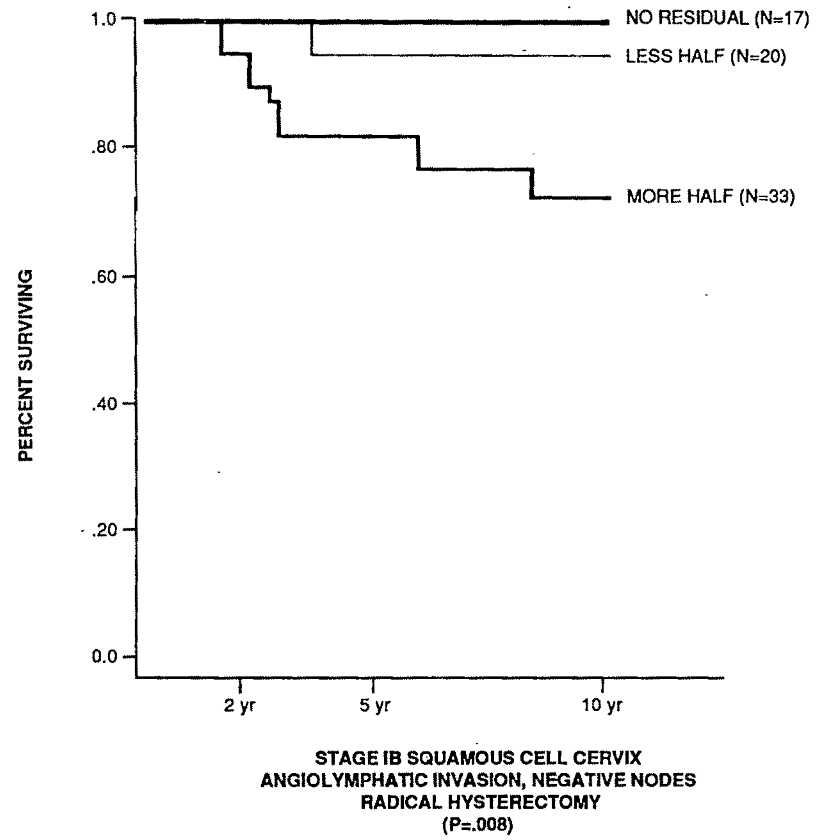

Fig. 8. Survival for patients undergoing radical hysterectomy with angiolymphatic invasion according to depth of cervical penetration, with negative lymph nodes.

tumors compared with 94% in well-differentiated or moderately differentiated lesions; these results are similar to ours. Chung et al.[15] also reported that patients with a poorly differentiated tumor had a higher incidence of recurrence and a lower 2-year survival.

Angiolymphatic invasion has been reported with variable results. Creasman et al.[3] reported that angiolymphatic invasion did not influence survival whereas Gauthier et al.[6] reported a decrease in survival from 92% to 83% when angiolymphatic invasion was present. Angiolymphatic invasion in the previously reported time period from our institution also influenced survival, although our recent survival of 88% is better than the 73% previously reported.[14] A previous report by White et al.[16] covering a 10-year time period from our institution reported no difference in survival. However, when a greater number of patients is analyzed in our present report, the survival for patients with angiolymphatic invasion decreases while the reported survival of 97% for patients without angiolymphatic invasion remains the same.

The lymph node status has been reported by all authors to significantly influence survival. Survival dropped to 60% in this report when all patients with positive lymph nodes were evaluated. The survival was also significantly influenced by the number of positive lymph nodes and decreased significantly when more than three nodes were involved. Survival also decreased when metastatic disease to regional lymph nodes was present bilaterally; however, all of these patients but one also had more than three positive lymph nodes. Thus these two factors oftentimes coexist.

The tumor size, lower uterine segment involvement, and depth of cervical penetration appear to significantly influence survival. Grimard et al.[7] reported that in stage IB disease the survival decreased from 90% to 50% when the lower uterine segment was involved. Gauthier et al.[6] evaluated risk groups and found the highest-risk group to have lesions >3 cm in size and deep cervical penetration. Underwood et al.[5] also found size and depth of invasion to significantly influence survival. Kishi et al.[8] evaluated the depth of invasion by determining the amount of fibromuscular stroma remaining at the paracervical margin. They reported an 8% mortality when 5 mm of thickness remained. The mortality increased to 26% when thickness was <3 mm and 57% when the margins were exposed. Creasman et al.[3] reported a 93% survival when only occult disease was present in the cervix; this rate is similar to that in our patients with no residual disease or <50% cervical penetration. The incidence of positive lymph nodes increases significantly with increasing depth of cervical invasion, and this factor was the major factor influencing survival in our Cox model.

Patients with stage IB squamous cell cancer of the cervix and poorly differentiated tumors, node metastases, deep cervical penetration, lower-segment involvement, or angiolymphatic invasion are at a higher risk for recurrence. As active chemotherapeutic agents are identified, these high-risk groups should be considered for adjuvant therapeutic trials.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Christopher J. Jolles, Salt Lake City, Utah. The authors state that the purpose of their retrospective study is to define clinical and pathologic features that predict recurrence of cervical cancer and enable them to select patients for adjunctive postoperative therapy. It is hoped that most high-risk patients are identified before radical hysterectomy; however, the clinician may use the available data in the selection of patients for radiotherapy after surgical exploration.

A major contribution of this series is the authors' confirmation of the findings of Kishi et al.[1] that the fraction of cervical invasion is a critical determinant of survival. Indeed they state that the amount of cervical stromal invasion was a more important determinant of survival than tumor size and lymph node status, although significant correlation was found between these features. It makes some sense that the fraction of cervix involved with tumor is an important factor. As the tumor involves greater fractions of cervical stroma, it gains access to the larger lymphatic trunks, which insert on the periphery of the cervix and probably serve as metastatic conduits. Given two tumors of equal size, one occurring in a small, primiparous or atrophic cervix and another in a large, grandmultiparous cervix, one can accept that the likelihood of metastatic spread is different.

As a logical extension of this work, I would make the observation that the current generation of magnetic resonance scanners are often able to visualize cervical tumors in many anatomic planes and distinguish them from uninvolved cervical stroma. One could thereby determine an approximate fraction of cervical stromal invasion without surgery and potentially develop radiographic criteria that would help the clinician determine the optimal treatment modality for an individual with early cervical cancer.

Before we apply these data in the clinic, we must be assured that possible sources of bias are not present. It should be recognized that the fraction of cervical stroma invaded was frequently determined in the radical hysterectomy specimen after conization, an operation that presumably removes a significant portion of tumor and thereby factitiously decreases the observed extent of cervical invasion. I would ask the authors how the data would be altered if the preconization tumor thickness were used in patients who underwent conization?

It is concerning that, unlike in previous reports, the presence of nodal metastases was not the most important determinant of survival. In this series the status of lymph nodes was established surgically. I note that lymph node status was known in 261 patients, yet only 213 underwent radical hysterectomy. What were the findings and clinical outcomes of the 48 patients who underwent exploration but who did not undergo radical surgery? Why was surgery not completed? If we knew the percentage of stromal invasion present in these cases, would it strengthen the authors' assertion that percentage of stromal invasion is as important a survival determinant as nodal metastasis? It is stated in the manuscript that the addition of radiation for high-risk indications did not influence survival in this series, but this issue was not rigorously examined and remains an important question that merits further prospective study.

I was intrigued by an unusual aspect of the patients described. A "normal Papanicolaou smear" had been obtained within one year of diagnosis in almost half of the patients. It has been observed that in well-screened populations the fraction of patients with cervical cancer who had recently had a negative Papanicolaou smear has increased significantly over the past several years, yet this number seems rather excessive and represents a most important and concerning contribution of their study. Have these Papanicolaou smears been reviewed? In the authors' opinion does this reflect a true change in the behavior of the disease, or does it reflect remediable clinical error on the part of the cytopathologist or the gynecologist?

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Dr. Howard W. Jones III, Nashville, Tennessee. This is an extensive review of the factors influencing prognosis in women with stage IB squamous cell carcinoma of the cervix. Their overall 5-year survival rate of 89% is excellent, and the 17% incidence of pelvic lymph node metastases in their series indicates that this is a representative sample of patients with stage IB disease. It is of interest that the survival rates reported here for patients treated from 1970 through 1985 are almost indentical to the results reported by Drs. Morley and Seski[1] for patients treated at the University of Michigan between the years 1945 and 1975. In that report adenocarcinoma of the cervix accounted for an increasing percentage of stage IB—from 3% during the first 10 years to 11.9% for the last 10-year period. This group also had a significantly worse prognosis, a 62% 5-year survival compared with 91% for the squamous cell lesions. Other authors also have noted a relative increase in the incidence of cervical adenocarcinoma, but most recent series[2] have shown no real difference in survival. You have restricted your report to squamous cell lesions, but I wonder if you have additional information concerning the patients with stage IB cervical adenocarcinoma?

Using the Cox statistical model, you found that the most significant prognostic characteristic was depth of cervical involvement by tumor. It is not clear to me exactly how this depth was measured or how those patients who had previously undergone cone biopsy were handled. In an excellent study Burghardt and his colleagues[3] from Graz, Austria, studied giant sections of 325 women treated surgically for cervical carcinoma. Using these sections, which included cross sections of the entire cervix and adjacent parametria, they were able to measure the area of the cervix and the area of the tumor. By this very exacting technique, they showed a 97.5% 5-year survival for women with <20% of the cervical area involved by tumor. This was statistically better than that of those women who had >20% involvement, in whom the survival rates were between 71% and 78%. It is somewhat surprising that in the authors' study cervical tumor invasion was a more significant indicator of prognosis than lymph node metastasis.

Finally, the authors said very little about their management protocol for women with stage IB cervical cancer. Did the type of therapy influence prognosis? What factors were used to select either radiation or radical surgery for their patients? How were patients with positive nodes managed?

While I congratulate the authors on this extensive work, I was disappointed not to find any new insights into the prognostic characteristics or management of women with stage IB squamous cell carcinoma of the cervix.

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DR. HOPKINS (Closing). I thank Dr. Jolles and Dr. Jones for their discussions. In regard to some of the questions, in the patients that underwent exploration for radical hysterectomy but in whom the procedure was not completed, the survival in that group was approximately 50%. Most of the time the radical hysterectomy was not done because of extremely high-risk features, such as large matted lymph nodes or common iliac periartic lymph nodes being diseased.

One or two of the patients had disease extension beyond the cervix at the time of exploration. That was a feature that did significantly influence survival when we looked at it.

None of the cytologic smears were reviewed. Most of them were not available for review, and what we looked for was whether the patients had reported a normal Papanicolaou smear within a year. So these patients had all been to a gynecologist, had been screened, and had negative reports. I think that would be a separate study on the false-negative rate of cytologic testing.

This article picked up on the previous report by Dr. Morley and Dr. Seski. In that report they gave the survival through 1970. The period 1970 through 1975 was not used for survival analysis in that report, so we went to the next 15 years, from 1970 to 1985, and reported that survival. Those 5 years from 1970 to 1975 include patients that we used for this survival analysis; they had not been used before.

During the same time period, we previously reported on adenocarcinoma of the cervix. Survival in that group was approximately 60% for stage I.

As to the measurement and depth of the cervical penetration, that's a difficult measurement to get. In patients who have radiation, obviously that depth cannot be ascertained. We used radical hysterectomy specimens and the cone biopsy specimens. Most of the time we had the radical hysterectomy specimen to use, and we measured from the squamous columnar junction over the site where the tumor began. We measured the entire cervix. We did not report as precisely as 20% but designated them as no tumor left in the cervix, <50% left, and >50% left.

The depth of invasion was a significant predictor of lymph node metastases, and that's why it became so important in the Cox model. Patients with deep penetration were more likely to die of the disease. The chances of having lymph node metastases were far greater for those patients where the depth was >50%.

We did look at the therapy but did not present our findings. The survival was equal in the patients who had radical surgery and those who had radiation therapy. So those data are similar to the findings previously reported by Dr. Morley.

Hysterosalpingography with color Doppler ultrasonography

Albert J. Peters, DO, and Carolyn B. Coulam, MD
Indianapolis, Indiana

To assess the accuracy of the diagnosis of tubal occlusion with the use of color Doppler flow ultrasonography and hysterosalpingography, 129 infertile women were studied. All 129 women had the procedure performed with an ATL Ultramark 9 (Advanced Technology Laboratories, Bothel, Wash.) color Doppler ultrasonography machine. Eighty-five of the 129 women also had an additional study including x-ray hysterosalpingography and/or chromopertubation. Of these 85 women, 58 had pelviscopic examination with chromopertubation. The frequency of diagnosis of tubal occlusion was compared among the three methods. When results of ultrasonography-hysterosalpingography were compared with those of x-ray hysterosalpingography and/or chromopertubation, 69 of 85 (81%) studies showed agreement, and 50 of 58 (86%) ultrasonography-hysterosalpingography findings agreed with observations at chromopertubation. The frequency of comparable findings between x-ray hysterosalpingography and chromopertubation is 75%. These data suggest that ultrasonography-hysterosalpingography is at least as accurate as x-ray hysterosalpingography in diagnosing tubal occlusion. In addition, ultrasonography-hysterosalpingography is safer and more cost-effective than x-ray hysterosalpingography and chromopertubation. (AM J OBSTET GYNECOL 1991;164:1530-4.)

Key words: Ultrasonography, hysterosalpingography, x-ray hysterosalpingography, tubal occlusion, chromopertubation

Tubal factors account for approximately 25% of the causes of infertility among three million couples in the United States.[1, 2] The diagnosis of tubal factor traditionally has been made by insufflating the fallopian tubes. In 1914, Rubin[3] described the use of the first attempt to assess tubal patency. Since that time a variety of techniques to demonstrate tubal patency have been used, including x-ray hysterosalpingography, chromopertubation at the time of laparoscopy, and radionuclide hysterosalpingography.[4] The most frequently used procedures to evaluate tubal patency currently are x-ray hysterosalpingography and chromopertubation. These procedures require the use of radiopaque dye, radiation, general anesthesia, and an operation. Ultrasonographic color Doppler flow does not require any of these risks. Therefore the accuracy of ultrasonographic color Doppler flow used to perform hysterosalpingography was studied.

Material and methods

One hundred twenty-nine women undergoing evaluation for infertility were studied for the presence or absence of tubal patency with ultrasonography-hysterosalpingography. The procedure was performed by instilling 5 to 50 ml of sterile saline solution transcervically through an H-S catheter (Ackrad Laboratories, Inc., Cranford, N.J.) into the endometrial cavity. Direction and flow into and out of the fallopian tubes were observed with color Doppler flow ultrasonography on an ATL Ultramark 9 (Advanced Technology Laboratories, Bothel, Wash.). Approximately half of the patients underwent Doppler scans transabdominally and one half transvaginally. When no fluid was observed to pass through the fallopian tubes or when no fluid was seen to enter the peritoneal cavity, tubal occlusion was diagnosed. The presence or absence of tubal occlusion diagnosed by ultrasonography-hysterosalpingography was compared with the results from x-ray hysterosalpingography and/or chromopertubation in the same woman. Of the 129 women who had ultrasonography-hysterosalpingography performed, 85 also had x-ray hysterosalpingography and/or chromopertubation procedures. Fifty-eight of these 85 women underwent pelviscopic examination with chromopertubation. In the remaining 71 patients, evaluation was not yet completed and laparoscopy had not been performed. The results of ultrasonography-hysterosalpingography were compared with the findings of x-ray hysterosalpingography and chromopertubation. In most cases x-ray hysterosalpingography had been performed before the patient's presentation to the Center for Reproduction and Transplantation Immunology. Two patients required

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6/6/29070

Table I. Ultrasonography-hysterosalpingography—Comparison with other tests for tubal patency

		Finding			
	No.	*Bilateral patency*	*Bilateral occlusion*	*Unilateral patency*	*Correlation (No.)*
Total No. of patients	129	85	20	24	—*
US-HSG compared to CPT and/or x-ray HSG	85	48 (70%)	9 (13%)	12 (17%)	69 (81%)
US-HSG compared to CPT alone	58	33 (66%)	12 (24%)	5 (10%)	50 (86%)

US-HSG, Ultrasonography-hysterosalpingography with Doppler flow; *CPT,* chromopertubation; *X-ray HSG,* x-ray hysterosalpingography.

*All 129 patients did not have two or more procedures for comparison.

a sedative before the procedure. None required antimicrobial treatment. No post-procedure infections occurred.

Results

Table I summarizes the findings of ultrasonography-hysterosalpingography from 129 women undergoing infertility evaluation. Bilateral tubal patency was diagnosed in 66%, unilateral patency in 16%, and bilateral tubal occlusion in 18%.

When the results of ultrasonography-hysterosalpingography were compared with those of x-ray hysterosalpingography and/or chromopertubation, 69 of 85 (81%) studies showed agreement, that is, patency or occlusion of the fallopian tube (Table II). Fifty of 58 (86%) ultrasonography-hysterosalpingography findings agreed with observations at chromopertubation at the time of laparoscopy (Table I). When ultrasonography-hysterosalpingography findings of each tube were compared with those of chromopertubation at the time of pelviscopic examination, 81% sensitivity and 94% specificity were calculated by the use of χ^2 analysis. The false-negative and false-positive rates for ultrasonography-hysterosalpingography in this group of patients were 19% and 6%, respectively. There were eight instances (14%) in which the ultrasonography-hysterosalpingography findings did not correlate with those of chromopertubation. In each of these instances unilateral tubal occlusion was diagnosed by one of the methods. Two studies revealed bilateral tubal patency on ultrasonography-hysterosalpingography whereas chromopertubation showed unilateral patency. Two other studies showed unilateral tubal patency with ultrasonography-hysterosalpingography, one of which revealed bilateral tubal patency and the other bilateral tubal occlusion at the time of laparoscopy with chromopertubation (Table II).

When x-ray hysterosalpingography was compared to chromopertubation in this group of patients, 22 of 31 (71%) studies correlated at the time of operation. The sensitivity and specificity of x-ray hysterosalpingography in this study were 65% and 82%, respectively. The false-negative and false-positive rates of x-ray hysterosalpingography in this group of patients were 45% and 18%, respectively. This difference in correlation between ultrasonography-hysterosalpingography and chromopertubation (86%) and x-ray hysterosalpingography (71%) is not significant ($p = 0.7$).

Table II. Noncorrelation between patency by ultrasonography-hysterosalpingography and by chromopertubation

Ultrasonography-hysterosalpingography	*CPT*	*No.*
Bilateral	Unilateral	3
Unilateral	Bilateral	4
Unilateral	0	1
TOTAL		8

Comment

Presented in this study is a new technique to assess fallopian tube patency. When this technique was compared with the more traditional techniques of assessing tubal patency, it was found to correlate well with the diagnosis of tubal occlusion. The frequency of tubal occlusion diagnosed by ultrasonography-hysterosalpingography correlated with x-ray hysterosalpingography and/or chromopertubation 81% of the time. When the results of ultrasonography-hysterosalpingography are compared with those of chromopertubation alone, a correlation existed 86% of the time. The diagnosis of tubal occlusion made with x-ray hysterosalpingography correlated with chromopertubation 71% of the time (Table III).[5-7] This value was similar to other comparisons in the literature.[5, 6] If chromopertubation is considered the gold standard for diagnosing tubal occlusion, ultrasonography-hysterosalpingography findings are at least as accurate as or more accurate than x-ray hysterosalpingography results in demonstrating tubal occlusion.

Table III. Comparison of x-ray hysterosalpingography to chromopertubation to assess tubal patency

Series	No. of procedures		Correlation
	Total	Correlation	
Keirse and Vanderwellen[5]	50	38	76%
Servy and Tzingounis[6]	97	74	77%
This study	31	22	71%
TOTAL	178		75%

In all instances in which the ultrasonography-hysterosalpingography findings did not correlate with the chromopertubation results, discrepancies involved a diagnosis of unilateral patency. We are now recommending repeating ultrasonography-hysterosalpingography when unilateral tubal occlusion is observed before proceeding with laparoscopy. The results of this study will help clarify the meaningfulness of the finding of unilateral patency at the time of hysterosalpingography, as well as define the frequency of unilateral tubal occlusion in infertile women.

In addition to the enhanced efficacy of ultrasonography-hysterosalpingography in diagnosing tubal occlusion, other advantages exist. No anesthetics, antimicrobials, or contrast dyes are necessary. The only agent used is sterile saline solution; therefore the risk of adverse reactions is minimized. There is also no exposure to radiation. The procedure is performed in the office setting, which makes it more convenient for both patient and physician. Costs are less. At our institution the cost to the patient for x-ray hysterosalpingography is approximately $400. The cost of ultrasonography-hysterosalpingography is approximately $250.

Difficulty in making a diagnosis of tubal occlusion arises in those patients with dilated hydrosalpinges because flow through the dilated fallopian tube may simulate spillage on the Doppler ultrasonography screen. In addition, tubal architecture is not demonstrated with Doppler flow hysterosalpingography as it is with x-ray hysterosalpingography. However, a recent study has shown this information is not useful in preoperative salpingoplasty procedures.[8]

Ultrasonography-hysterosalpingography is a new technique to assess fallopian tube patency that can easily be performed in an office setting with minimal discomfort, low cost, and high accuracy when compared to the more traditional techniques.

In conclusion, this study represents results of the use of color Doppler flow ultrasonography to assess fallopian tube patency in the infertile patient. The procedure is as good as x-ray hysterosalpingography in evaluating tubal patency when both techniques are compared to chromopertubation at the time of laparoscopy. Other advantages such as lower cost, convenience, lack of exposure to radiation, no anesthetics, and no operative complications are attractive. Disadvantages include possible false-positive results of patency when the patient with a hydrosalpinx is evaluated. As more patients are evaluated with this technique, its place in the investigation of the infertile couples will be established.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. John F. Randolph, Ann Arbor, Michigan. The search for an easier, cheaper, and more convenient method for assessing the anatomy of the upper female genital tract in the evaluation of the infertile couple has not produced a more promising technique than the one described by the authors. With the increasing availability of real-time ultrasonography in the offices of physicians treating couples with infertility, the availability of color Doppler ultrasonography offers the possibility of an immediate assessment of upper genital tract anatomy on presentation of the patient. This new technique takes advantage of the technologic evolution of an idea initially investigated in the early 1980s.

I had the privilege of conducting a similar study[1] with an earlier generation of ultrasonographic equipment and encountered a number of difficulties pertinent to the study under discussion. In that study 61 women underwent real-time transabdominal pelvic ultrasonography during the instillation of saline solution into the uterus after being anesthetized in preparation for surgical evaluation of the pelvis. With the equipment available at that time patency of the fallopian tubes could easily be demonstrated by the accumulation of fluid in the cul-de-sac, but flow through the tubes was difficult to determine. Therefore the patency or occlusion of an individual fallopian tube was often undetermined. Color Doppler ultrasonography presents a more sensitive measure of flow through the tubes and therefore makes the individual tubal patency more measurable.

It also became clear in our earlier study that assessment of a new technique required a careful comparison with an established gold standard to determine sensitivity and specificity. By use of such measurements the inherent properties of a test or technique can be measured independently of a particular patient population and allow meaningful evaluations of the technique in various clinical studies.

The authors presented data to support the hypothesis that ultrasonography-hysterosalpingography was at least as accurate as x-ray hysterosalpingography in assessing tubal patency. Unfortunately, the data as presented are incomplete and therefore do not allow an accurate analysis of the study. It is not obvious from the data how many patients had undergone x-ray hysterosalpingography or how many patients had undergone all three procedures. The ideal comparison of ultrasonography-hysterosalpingography to x-ray hysterosalpingography with chromopertubation at the time of operation as the gold standard should be made in patients who had all three procedures performed. If the assessment is limited to patients having chromopertubation only as compared to ultrasonography-hysterosalpingography, then values for sensitivity and specificity can be calculated. However, side-specific patency is not indicated and becomes a critical omission for the determination of the properties of the new technique.

In particular, five patients were listed as demonstrating unilateral patency with both techniques. Without an indication of which tube was patent with each technique it is impossible to determine if the tests were in complete agreement or complete disagreement for any individual patient. On the basis of the possible extremes, the calculated sensitivity for ultrasonography-hysterosalpingography versus chromopertubation ranges from 89% to 95% and the specificity from 74% to 88%.

Additionally, the authors concluded that ultrasonography-hysterosalpingography is safer and more cost-effective than x-ray hysterosalpingography or chromopertubation. With a generally accepted infectious morbidity rate of about 1% after x-ray hysterosalpingography, it is premature to conclude a lower rate of infectious morbidity with only 129 women studied, although the risks of ionizing radiation, iodinated contrast materials, general anesthesia, and operative endoscopy are avoided. Moreover, without cost figures for comparison and with the significant expense of color Doppler ultrasonographic equipment, it is not possible to establish definite cost savings.

I beseech the authors to please provide us with more complete data with which to evaluate this exciting new advance. Intuition urges us to support their conclusions, but the data do not.

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Dr. Rudy E. Sabbagha, Chicago, Illinois. This article examines the accuracy of diagnosing fallopian tube patency by color Doppler ultrasonography. In the study only 58 of 129 infertile women were selected for examination by both ultrasonography-ultrahysterosalpingography and laparoscopic chromopertubation. However, the selection criteria were not described and the outcomes for the remaining 71 patients were not detailed. Similarly the selection criteria for ultrasonographic and x-ray imaging were not presented.

In the study the correlation between ultrasonography and laparoscopy (86%) appears similar to that between x-ray hysterosalpingography and chromopertubation (71%). However, these differences were not compared statistically and the results fall in the realm of impressions. A number of advantages pertaining to color Doppler ultrasonography also were listed, including lack of radiation, possible decrease in the use of pelviscopic examination with the patient under general anesthesia, and cost-effectiveness. However, the latter 2 statements remain unconfirmed.

Specifically, pelviscopic examination provides the gynecologist with information in addition to tubal patency, including presence of uterine myomas or anomalies, tubal dilation or adhesions, or both, and endometriosis. Thus without a blinded prospective study it may not be possible to conclude that ultrasonography will translate into a decrease in the use of pelviscopic examination with the patient under general anesthesia.

As to cost-effectiveness of ultrasonography, the authors did not supply us with data comparing the cost of ultrasonography to x-ray hysterosalpingography. In such comparisons one has to consider that each ultrasonographic machine with color Doppler capability comes with a price tag of approximately $180,000. When indirect cost is factored in with equipment depreciation, as well as with physician and allied health ultrasonographer time, the cost may not be different from that of x-ray hysterosalpingography.

Finally, although the authors quoted a reference that dismisses any benefit to the evaluation of tubal architecture and dilation by x-ray hysterosalpingography, many gynecologists find that the overall information gathered by this x-ray method is important. This includes identification of uterine anomalies and/or distortion of the uterine cavity by submucous or intramural myomas. Certainly, the uterus, including the uterine cavity, also can be evaluated by vaginal ultrasonography. However, such evaluation is likely to drive the cost of the procedure upward.

The authors have shown an interesting application of new technology—color Doppler ultrasonography. However, additional data are necessary to define its precise role and its possible advantages.

Dr. L. Russell Malinak, Houston, Texas. I would like to ask the authors how many patients were nulligravid and how many were parous. It seems that a 1 ml balloon in the uterus of a nulligravid woman might be associated with pain and consequent spasm of the uterus and fallopian tubes. How painful was the procedure?

Is there an advantage in using Doppler equipment over a standard ultrasonography machine, and can you visualize peritubal adhesions, as in hysterosalpingography?

Dr. Peters (Closing). Thank you for your thoughtful comments and your questions. We did look at sensitivity and specificity and found an 89% sensitivity along with a 90% specificity.

Regarding Dr. Sabbagha's question concerning patient selection, these patients presented to Dr. Coulam's practice with the complaint of infertility and they had fulfilled the standard criteria of infertility. They had already undergone one form of assessment of tubal patency.

Dr. Sabbagha also brought out the point of possibly trying to replace pelviscopic examination. The intent was not to replace pelviscopy, just as x-ray hysterosalpingography does not replace pelviscopy, but simply to give us another mode of studying the fallopian tube. Indeed, if patency can be assessed equally well with ultrasonographic hysterosalpingography, it may possibly give x-ray hysterosalpingography some competition.

However, we did not intend to omit pelviscopic examination because, as suggested, many things can be found at the time of pelviscopic examination that could be critical to the infertile patient.

As to the cost issue, yes, indeed, Doppler ultrasonography machines are very expensive. I would suppose and hope that with time the cost of those machines will decrease, as with most new technologies.

As far as cost per procedure, the cost of this particular procedure is approximately $165 in our institution, which is the cost of the scan. In our institution the cost of x-ray hysterosalpingography is close to $300. So there is a significant cost saving there.

As to Dr. Malinak's question, I'm sorry, I don't know the exact percentage of patients that were primigravid or multigravid. However, I did not have difficulty maintaining the balloon within the uterine cavity. As I stated in the advantages, most of the patients claimed that they had less discomfort than with x-ray hysterosalpingography.

We cannot provide, at least with our skill, at this point an adequate assessment of peritubal disease.

Dr. Randolph brought up the point of sensitivity and specificity that I did address.

I was not referring to safety in the sense that the radiation was so great that it would harm the patient. I was referring to the allergic types of reactions that some patients encounter with the contrast dyes, which are eliminated simply because we're using saline solution.

Comparison of associated high-risk factors and perinatal outcome between symmetric and asymmetric fetal intrauterine growth retardation

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This study compares associated high-risk factors and perinatal outcome between 273 symmetric and 445 asymmetric infants with intrauterine growth retardation. No differences were seen in 17 obstetric, medical, and environmental-behavioral high-risk factors between symmetric and asymmetric intrauterine growth retardation. In preeclampsia, the incidence of symmetric intrauterine growth retardation is higher than that of asymmetric intrauterine growth retardation. The timing of the interaction between the high-risk factor and the stage of gestation is more important than the specific high-risk factor in determining whether symmetric or asymmetric intrauterine growth retardation is produced. On comparison of perinatal outcome between the two groups, we concluded: (1) that the onset of symmetric intrauterine growth retardation occurs much earlier in the course of pregnancy than does asymmetric intrauterine growth retardation, (2) that more symmetric than asymmetric pregnancies with intrauterine growth retardation result in preterm delivery, (3) that the neonatal morbidity rate for symmetric intrauterine growth retardation is higher than that for asymmetric intrauterine growth retardation, and (4) that term symmetric infants with intrauterine growth retardation tend to have a lower mean birth weight and a higher incidence of small placentas than term infants with asymmetric intrauterine growth retardation. (AM J OBSTET GYNECOL 1991;164:1535-42.)

Key words: Symmetric IUGR, asymmetric IUGR, associated high-risk factors, perinatal outcome

Intrauterine growth retardation (IUGR) is recognized as an important cause of low birth weight, second only to the problem of preterm birth.[1] The IUGR infant is associated with multiple causative factors[2, 3] and with high levels of perinatal mortality and morbidity.[4-7] Two types of IUGR infants have been identified clinically: symmetric and asymmetric.[3, 6, 7] The purpose of this study was to compare associated high-risk factors and perinatal outcome between symmetric and asymmetric IUGR infants.

Patients and methods

Between July 1979 and December 1985, 718 IUGR infants among a total of 22,616 infants were delivered at the Chicago Lying-In Hospital, The University of Chicago. During the study period, all patients with high-risk factors were enrolled prenatally in either the Obstetric High-Risk Clinic or the Medical High-Risk Clinic. Historic high-risk factors were recorded during the initial visit, and high-risk factors that developed during gestation were identified in subsequent clinical visits. Clinical data for all high-risk patients during pregnancy were entered into the University of Chicago OB-File computer system whenever a high-risk factor was identified. High-risk patients then had close follow-up, with intervals of 1 to 2 weeks at our high-risk clinics. Clinical data of low-risk obstetric patients during pregnancy were not entered into the computer system. However, clinical data of labor and delivery and newborn data for both high- and low-risk patients who were delivered at our institution were entered into the computer system. During the study period, 3788 patients had prenatal care at our high-risk clinics.

Clinically suspected IUGR cases, such as those with retarded uterine fundal height, were further evaluated by serial ultrasonographic measurements to establish the diagnosis of suspected IUGR. Multiple static axial and longitudinal images of the fetus and uterus were taken during each ultrasonographic examination. Each examination included fetal measurements such as biparietal diameter, head circumference, abdominal circumference, femur length, calculation of head circumference/abdominal circumference ratio, and estimated fetal weight. Each patient also was screened for gross fetal anomalies and quantitative amniotic fluid volume.

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Table I. Comparison of incidences of various high-risk factors between symmetric and asymmetric IUGR*

High-risk factor	*Symmetric*	*Asymmetric*	χ^2 *Test*
Total cases	273	445	
Demographic-behavioral risk factor			
Primigravid	64 (23.4%)	100 (22.4%)	p = NS†
Age <17 yr	24 (8.8%)	26 (5.8%)	p = NS
Age >35 yr	8 (2.9%)	12 (2.7%)	p = NS
Smoking	77 (28.2%)	136 (30.6%)	p = NS
Alcohol	24 (8.8%)	37 (8.3%)	p = NS
Drug abuse	28 (10.3%)	33 (7.4%)	p = NS
Obstetric risk factor			
Preeclampsia	52 (19.0%)	55 (12.4%)	$p < 0.025$
Twin gestation	24 (8.8%)	36 (8.1%)	p = NS
Poor obstetric history	39 (14.3%)	44 (9.9%)	p = NS
Poor weight gain	40 (14.7%)	70 (15.7%)	p = NS
Antepartum hemorrhage	11 (4.0%)	15 (3.4%)	p = NS
Medical risks in current pregnancy:			
Chronic hypertension	25 (9.2%)	28 (6.3%)	p = NS
Anemia (hemoglobin <10 gm/dl)	44 (16.1%)	82 (18.4%)	p = NS
Urinary tract infection (symptomatic)	14 (5.1%)	36 (8.1%)	p = NS
Asthma	11	14	
Hemoglobinopathy	8	17	
Cardiac disease	5	6	
Diabetes mellitus	1	12	

*Each case of IUGR may have one or more high-risk factors.

†Power of statistics for 13 items with p = NS. We had a 7% chance of detecting a 20% difference in the groups.

A fetus with two abnormal results among the three parameters, such as abdominal circumference of <10th percentile, with a head circumference/abdominal circumference ratio of >2 SD above the mean, (e.g., 1.21 at 34 weeks, 1.05 at 40 weeks) or an estimated fetal weight <10th percentile was diagnosed as having suspected IUGR. Ultrasonographic evaluation, however, was not performed routinely for all of our obstetric patients, particularly during the first half of the study period, 1979 to 1982. Prenatally suspected IUGR cases were subjected to a weekly high-risk clinic visit and a nonstress test. A nonreactive nonstress test was followed by a contraction stress test,[8] a biophysical profile scoring evaluation,[9] or both. Antenatally suspected IUGR pregnancies were terminated at 38 weeks of gestation after documentation of fetal lung maturity. Earlier termination was carried out either for fetal indications or because of maternal deterioration, consistent with our management protocol.[10]

During labor and delivery, various signs of fetal distress, including abnormal fetal heart rate patterns, meconium-stained amniotic fluid, and fetal acidosis, were intensively monitored. At the time of delivery all IUGR infants were evaluated by two pediatricians. All of the preterm IUGR infants in the study series received neonatal care at The University of Chicago's intensive care nursery. After the infant was discharged from the intensive care nursery, the medical record of each preterm IUGR neonate was carefully reviewed and various neonatal morbidities such as respiratory distress syndrome, neonatal infection, neonatal acidosis, hypoglycemia, and hyperbilirubinemia were identified.

At the conclusion of data collection, all 718 IUGR infants were assigned to one of two groups, symmetric or asymmetric IUGR. The symmetric IUGR group was defined as <10th percentile for both body weight and head circumference for the gestational age at birth. The asymmetric IUGR group was defined as a birth weight of <10th percentile but head circumference of >10th percentile for gestational age, on the basis of our own fetal growth curve, which has been in existence since 1983.[3] Preterm IUGR was defined as <10th percentile for birth weight with a gestational age of <37 weeks.

For a comparison of associated high-risk factors, 18 factors (including seven medical complications, five obstetric factors, and six environmental-behavioral factors) were compared between the two groups. Fourteen factors were compared statistically by the χ^2 test for their incidence in each group. Four other factors were not analyzed because of the small number of cases in each category. Further analysis was performed for the 57 IUGR infants whose mothers developed preeclampsia; all of these cases had at least 4 weeks of prenatal care before delivery.

Several parameters of perinatal outcome were compared between symmetric and asymmetric IUGR infants by means of either the t test or the χ^2 test for statistical analysis.

Results

Associated high-risk factors. The overall incidence of IUGR during the study period was 3.2% (718 cases of IUGR per 22,616 total births). Among the 718 IUGR infants, 273 (38%) were symmetric and 445 (62%) were asymmetric IUGR according to the criteria mentioned previously. Approximately 80% of symmetric IUGR (217/273) and 70% of asymmetric IUGR (312/445) had one or more identifiable high-risk factors.

A comparative analysis of the 18 associated risk factors is shown in Table I. The incidence of each of the following risk factors was not statistically different between symmetric and asymmetric IUGR: chronic hypertension, anemia, symptomatic urinary tract infection, twin gestation, poor obstetric history, poor weight gain, antepartum hemorrhage, maternal age <17 years, smoking, use of alcohol, and drug abuse. For those risk factors with a small number of cases in each category, such as asthma, hemoglobinopathy, cardiac disease, diabetes mellitus, and maternal age >35 years, there was no difference in the incidence of symmetric and asymmetric IUGR. Preeclampsia was the only high-risk factor that exhibited a higher incidence of symmetric than asymmetric IUGR (19% vs 12%, $p < 0.025$).

Table II compares symmetric and asymmetric IUGR infants whose mothers had preeclampsia before delivery. The mean gastational age at the diagnosis of IUGR, the mean gestational age at delivery, and the mean birth weight all were significantly lower in the symmetric IUGR group when compared with the asymmetric IUGR group. Most symmetric IUGR infants were delivered of women with either early onset of moderate to severe preeclampsia or chronic hypertension with superimposed preeclampsia.

One symmetric and 12 asymmetric IUGR infants were born to diabetic mothers. Seven of the 12 asymmetric IUGR infants were the products of mothers with juvenile diabetes (three with class C, three with class D, and one with class F diabetes).

Antenatal diagnosis of IUGR. Approximately one third of the IUGR cases in the study were identified clinically and confirmed by ultrasonography during pregnancy in both groups (93 cases or 34.0% in the symmetric group and 134 cases or 30.1% in the asymmetric group, p = NS). Among IUGR fetuses diagnosed antenatally, the difference in the timing of clinical detection between symmetric and asymmetric IUGR was quite striking: before 32 weeks, 23% (21 cases) symmetric versus 2% (3 cases) asymmetric ($p < 0.001$, χ^2 test); at 34 weeks, 46% (43 cases) symmetric versus 14% (19 cases) asymmetric ($p < 0.001$, χ^2 test); and at 36 weeks, 82% (76 cases) symmetric versus 64% (86 cases) asymmetric ($p < 0.01$, χ^2 test). Fig. 1 illustrates cumulative curves of the incidences of clinical detection of the two types of IUGR according to gestational age.

Preterm delivery, birth weight, and placental weight. As shown in Table III, the incidence of preterm delivery was higher for symmetric than for asymmetric IUGR (32.6% vs 12.4%, $p < 0.001$).

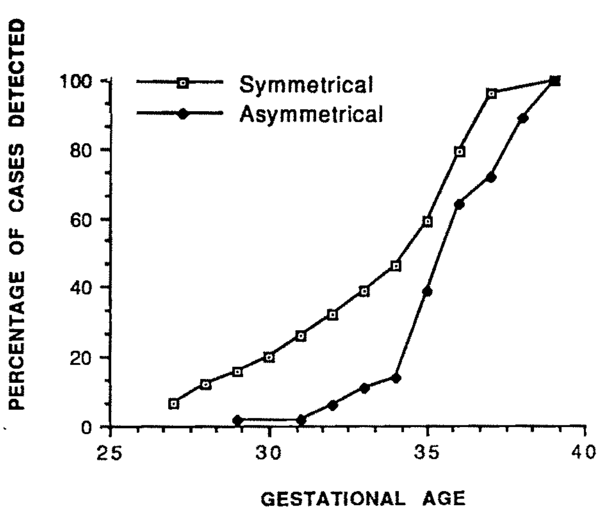

Fig. 1. Cumulative curves of incidences at time of clinical detection for symmetric and asymmetric IUGR according to weeks of gestation (see text for details).

Table II. Comparison between symmetric and asymmetric IUGR associated with maternal preeclampsia*

	Symmetric	*Asymmetric*	*t Test*
Total No. of cases	31	26	
Gestational week IUGR Diagnosed	29.5 ± 2.0	34.1 ± 1.5	$p < 0.001$
Gestational week at delivery	32.7 ± 3.1	36.7 ± 2.2	$p < 0.001$
Interval between diagnosis and delivery (wk)	3.2 ± 2.8	2.6 ± 2.2	p = NS
Birth weight (gm)	1300 ± 418	2050 ± 338	$p < 0.001$

*All patients had at least 4 weeks of prenatal care before delivery. All data expressed as mean ± SD.

Fig. 2 shows the mean birth weight according to gestational week for both symmetric and asymmetric preterm IUGR infants. For the purpose of comparison, a control curve of the mean birth weight by gestational week on the basis of a total of 3216 preterm non-IUGR infants delivered prematurely at out institution during the study period, ranging from 28 to 36 weeks, also is illustrated. As expected, the mean weight curves for both symmetric and asymmetric IUGR infants are far below the control curve. It is of interest to note that the mean weight curve for symmetric IUGR runs below that of asymmetric IUGR at each gestational week before 37 weeks.

Of those IUGR infants who were delivered at term (≥37 weeks' gestation), the mean birth weight of symmetric IUGR infants was significantly lower than that of asymmetric IUGR infants (2167 ± 260 vs 2385 ± 105 gm, $p < 0.001$ by t test). The incidence of small placenta at term gestation (defined as a placental weight of <400 gm) was significantly higher in symmetric than

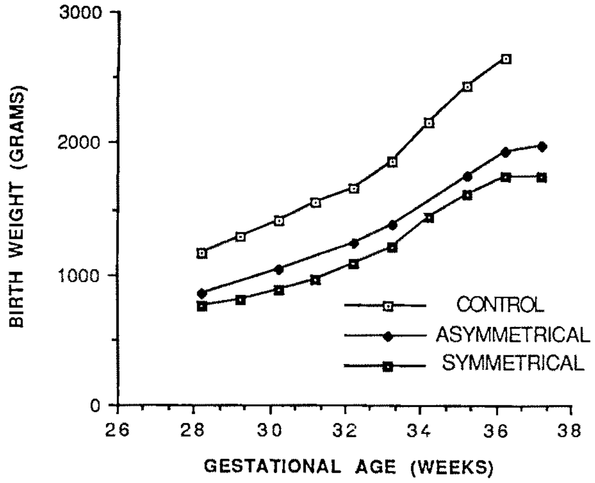

Fig. 2. Mean body weight by weeks of gestation for preterm symmetric and asymmetric IUGR. Control curve is obtained from mean birth weight data for our 3216 preterm appropriate-for-gestational-age population during the study period. Mean birth weight data were calculated week by week according to gestational age between 28 and 36 weeks.

Table III. Perinatal events and fetal outcome

	Symmetric	*Asymmetric*	χ^2 *Test*
Total No. of cases	273	445	
Preterm birth	89 (32.6%)	55 (12.4%)	$p < 0.001$
Total intrapartum fetal distress*	117 (42.9%)	153 (34.4%)	p = NS
Abnormal FHR with MSAF	54 (19.8%)	69 (15.5%)	p = NS
Abnormal FHR with pH <7.20	36 (13.1%)	23 (5.2%)	$p < 0.05$
Cesarean section	55 (20.1%)	71 (16.0%)	p = NS
Apgar score ≤6			
1 min	87 (31.9%)	91 (20.4%)	$p < 0.01$
5 min	43 (15.8%)	35 (7.9%)	$p < 0.05$
Neonatal death	24 (8.8%)	8 (1.8%)	$p < 0.001$
Fetal anomalies†	16 (5.9%)	13 (2.9%)	p = NS

FHR, Fetal heart rate; *MSAF*, meconium-stained amniotic fluid.

*Intrapartum fetal distress. All cases had abnormal fetal heart rate patterns, some of them combined with meconium-stained amniotic fluid and fetal acidosis.

†Two cases of chromosomal abnormalities and one case of cytomegalovirus in the symmetric group and three cases of chromosomal abnormalities in the asymmetric group.

in asymmetric IUGR (37.5% vs 12.6%, $p < 0.001$ by χ^2 test).

Perinatal events and fetal outcome. As shown in Table III, fetal outcome for symmetric IUGR was much worse than for asymmetric IUGR infants. There was a higher incidence of preterm delivery, depressed Apgar scores at both 1 and 5 minutes, fetal acidosis, and neonatal death. However, the incidences of total intrapartum fetal distress, cesarean delivery, and gross fetal anomalies were not significantly different between the two groups.

Neonatal morbidity among preterm IUGR infants. Neonatal morbidity among the 89 preterm symmetric IUGR and the 55 preterm asymmetric IUGR infants is compared in Table IV. The incidences of low 5-minute Apgar score, respiratory distress syndrome, neonatal infection, and neonatal acidosis were higher for symmetric than for asymmetric preterm IUGR infants. There were no significant differences between the two groups in the incidence of hyperbilirubinemia, hypoglycemia, or low 1-minute Apgar score.

Comment

An IUGR infant is commonly defined as one weighing <10th percentile in birth weight for gestational age.[11] However, because there is no standard population from which to derive these percentiles, the birth weights that serve as the cutoff point by various published studies may differ as much as several hundred grams at any gestational week during the third trimester.[12] This variability may result from population differences in sex, or race and from different geographic areas studied, as well as differences in study methods. In this study we used a fetal growth curve (constructed by Lowensohn and based on total live births between 1978 and 1982 at our institution) to define IUGR if the birth weight fell below the 10th percentile line for each gestational week (Fig. 3).[3] A comparison of the 10th percentile line of two different fetal growth curves shows a close similarity between the Chicago and the Colorado curve.[11] The 10th percentile growth curve from the state of California,[13] however, suggests that their newborns at the cutoff point for IUGR between 26 and 36 weeks of gestation are heavier than ours. When we used the Chicago curve to study a total of 7177 deliveries at our institution between January 1979 and May 1981, there were 426 IUGR newborn infants, an incidence of 5.9%.[3] In this study the incidence of IUGR was 3.2% according to the same study method in the same institution but during a different study period. The reason for this difference in the incidence of IUGR during two time periods is not well understood.

Among 718 IUGR infants in this study series, 189 (26.3%) were not associated with any high-risk factors. We believe that many of them, particularly those with symmetric IUGR, may belong to the group of so-called "constitutionally small" babies. On the basis of a four times increased risk of stillbirth, Myers and Ferguson[14] studied a population in the state of Illinois and argued that the 3rd percentile should be used to define IUGR in preterm gestations before 31 weeks, whereas the 15th to 17th percentile should be used to define IUGR

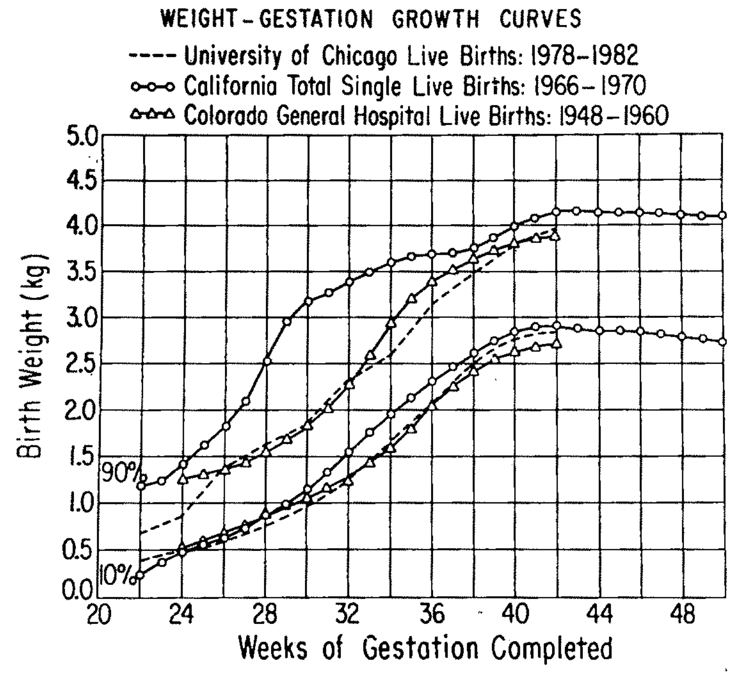

Fig. 3. Weight–gestational age fetal growth curves of the University of Chicago, state of California, and Colorado General Hospital. Both 10th and 90th percentile lines are shown. Note Chicago and Colorado 10th percentile lines are in close agreement. (Courtesy of R.I. Lowensohn. From Lin C-C, Evans MI, eds. Intrauterine Growth Retardation: Pathophysiology and Clinical Management. New York: McGraw-Hill, 1984:40. Reproduced with permission from McGraw-Hill, Inc.)

in those pregnancies approaching term gestation. In view of the heterogeneity of the various causes of IUGR, there are some inevitable limitations to using the 10th percentile of birth weight as the sole criterion for diagnosing IUGR.

Among six infants with chromosomal abnormalities and congenital viral infection, three cases were in the symmetric IUGR group and the other three cases were in the asymmetric IUGR group. In all three cases in the asymmetric group, the head circumference measurements were between the 10th and 25th percentiles. Using a definition of less than the 25th instead of the 10th percentile for head circumference to define symmetric IUGR would place all of these cases in the symmetric group. This idea is consistent with the findings of Sabbagha,[15] who reported an incidence of IUGR infants of >50% in cases associated with a small biparietal diameter (<25th percentile) in early pregnancy. However, if adopting a definition of <25th percentile for head circumference to differentiate symmetric from asymmetric IUGR would lead to an unrealistically high incidence of symmetric IUGR in our study population. If a criterion of <10th percentile for head circumference is used to define symmetric IUGR, the 38% incidence of symmetric IUGR in this study series is already somewhat higher than the 20% to 25% incidence of symmetric IUGR cited in the literature.[2, 3, 16] Obviously, this dilemma of whether to use the 10th or the 25th percentile for head circumference to define symmetric IUGR is difficult to resolve.

Table IV. Neonatal morbidity among preterm IUGR infants

	Symmetric	*Asymmetric*	χ^2 *Test*
Total No. of preterm births	89	55	
Apgar score ≤6			
1 min	57 (64%)	27 (49.1%)	p = NS
5 min	39 (43.8%)	11 (20.0%)	$p < 0.025$
RDS	44 (49.4%)	11 (20.0%)	$p < 0.025$
Neonatal infection	22 (24.7%)	8 (14.5%)	$p < 0.05$
Neonatal acidosis	20 (22.5%)	4 (7.3%)	$p < 0.025$
Hyperbilirubinemia	29 (32.6%)	13 (23.6%)	p = NS
Hypoglycemia	39 (3.4%)	5 (9.1%)	p = NS

RDS, Respiratory distress syndrome. Neonatal acidosis, pH <7.20. Hyperbilirubinemia, serum bilirubin level >10.0 mg/dl. Hypoglycemia, plasma glucose level <30.0 mg/dl.

Eighteen high-risk factors that my be attributed to the development of fetal growth retardation were found in 80% of symmetric and 70% of asymmetric IUGR in our study population. Our data are consistent with Galbraith et al.,[2] who found that two thirds of IUGR infants come from the population with risk factors. It is interesting to find that an almost equal incidence of symmetric and asymmetric IUGR infants were produced by pregnancies associated with such high-risk factors as chronic hypertension, anemia, antepartum hemorrhage, and twin gestation. These factors were previously thought to be related only to the production of asymmetric IUGR.[2,3,17] In addition, more symmetric than asymmetric IUGR infants were associated with preeclampsia. The majority of the preeclampsia cases in this study series, however, was cases involving either an early onset of preeclampsia (before 32 weeks of gestation) or chronic hypertension with superimposed preeclampsia. The extremely poor fetal outcome seen in severe cases of preeclampsia during midpregnancy and in chronic hypertension with superimposed severe preeclampsia has been well documented.[18,19] The preterm growth-retarded fetus so produced resembles symmetric rather than asymmetric IUGR in its appearance.

On the basis of the results of the present study, medical, obstetric, and environmental-behavioral high-risk factors do not produce predominantly symmetric or asymmetric IUGR infants. We speculate that the timing of the interaction between high-risk factors and the stage of gestation, rather than the nature of each high-risk factor, plays the more important role in determining whether symmetric or asymmetric IUGR will occur in human pregnancies.

Intrauterine fetal growth can be viewed as series of incremental changes in the size of the fetus, as well as in the function of various fetal organ systems, throughout the entire period of pregnancy. These changes are influenced by both genetic and environmental factors, which interact with cell proliferation, organ differentiation, and metabolic development in the process of fetal growth. On the basis of animal experiments and human observations, Rosso and Winick[17,20] have described three phases of fetal cellular growth. The first phase is cellular hyperplasia, which includes an increase in cell number occurring during the first 16 weeks of embryonic and fetal life. Fetal insult during this phase leads to the development of symmetric IUGR with the primary effect being a reduced cell number. These infants are usually small in size with a reduction in all external measurements, e.g., weight, length, and head circumference, thus producing a normal ponderal index. This type of growth retardation is frequently associated with congenital malformations. The second phase of fetal growth is concomitant hyperplasia and hypertrophic cellular growth. This phase encompasses the period from 16 to 32 weeks' gestation, during which time there is progressive decrease in the rate of cell hyperplasia and a progressive increase in hypertrophic cell growth. Fetal insult during this phase usually produces a mixed or intermediate type of IUGR with features resembling symmetric IUGR if the insult occurs earlier and asymmetric IUGR if the insult occurs in the latter half of this phase. The third phase of fetal growth extends from 32 weeks to term. During this time period, cell size rapidly increases, along with the rate of glycogen and fat deposition. An insult during this phase leads to the development of asymmetric IUGR with its relative brain-sparing phenomenon. This well-known hypothesis serves as a basis to support our speculation that the timing of the interaction between high-risk factors and the stage of gestation is far more important than the nature of each high-risk factor. Thus a symmetric IUGR fetus can be produced by the pregnant woman with an early onset of severe preeclampsia.

Approximately one third of both the symmetric and asymmetric IUGR cases in our study series were identified by prenatal examination and subsequent ultrasonographic examination during pregnancy. Using serial ultrasonography for prenatal detection of IUGR in a large study population in London, Warsof et al.[21] were able to detect IUGR with an accuracy of 68%. Hughey[22] was able to detect only 23% of IUGR fetuses by means of clinical methods and selective screening, but he was able to increase his IUGR detection rate to 57% by using routine ultrasonographic screening. The relatively low prenatal IUGR detection rate in this study series is mainly due to the fact that routine ultrasonographic screening is not performed on our entire obstetric population.

The major findings in the comparison of perinatal outcome between symmetric and asymmetric IUGR in this study series include: (1) that the onset of symmetric IUGR occurs much earlier in the course of pregnancy than that of asymmetric IUGR, (2) that more symmetric than asymmetric IUGR results in preterm delivery, (3) that the morbidity rate for symmetric IUGR is higher than that for asymmetric preterm IUGR neonates, and (4) that term symmetric IUGR infants tend to have a lower body weight and a smaller placenta than do term asymmetric IUGR infants.

Recently, investigators[23,24] have paid special attention to the preterm growth-retarded infant because, in general, infants in this category have the worst prognosis. In addition to the knowledge of a worse prognosis for preterm IUGR than for preterm appropriate-for-gestational-age infants, this study has further documented that the neonatal morbidity rate among preterm symmetric IUGR infants is significantly higher than that of preterm asymmetric IUGR infants. However, some of these differences in neonatal morbidity rates may be attributed in part to the difference in preterm birth rate observed between the two groups.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ALFRED B. KNIGHT, Temple, Texas. This article is a review of IUGR newborns in a data base of 22,616 patients delivered between 1979 and 1985. I have presumed in this discussion that information was collected on all patients in the data base.

There many complex issues regarding abnormal fetal growth yet to be answered, not the least of which is the definition.[1] The further classification of IUGR as symmetric or asymmetric was an attempt to associate cause and prognosis after the child was born with a gestational pathophysiologic condition. This concept could then be extended to in utero diagnosis. Lin and colleagues were unable to confirm the classic clinical association of certain diseases of fetus and mother with specific subtypes of growth retardation. Clarification of a number of issues relative to the patient data base and patient selection would be helpful before we discard two decades of elegant teaching of the pathophysiologic features of IUGR.

1. Dr. Lin and colleagues have used the definition of IUGR to be a birth weight for gestational age of <10th percentile. I would have anticipated that the number of infants identified would approach 10% of the data base, at least markedly greater than the 3.2% screened. Why is there such a low incidence of IUGR in their population?

2. The method for assigning gestational age was not elaborated in this manuscript and remains a critical element to the definition of IUGR. Both obstetric and neonatal information can be used, but neither can assign gestational age with a greater accuracy than plus or minus 2 or 3 weeks (excluding those patients with "stellar" dates). Perhaps an even greater error is introduced with Dubowitz gestational ages <34 weeks complicated by IUGR.[2] What method was used to determine gestational age?

3. Symmetric versus asymmetric IUGR is defined by a head circumference of >10% or <10%. Others in the newborn period have used the 25th percentile. The characterization of IUGR of differing symmetry reflects an attempt to understand and categorize causes of IUGR. In fact, rarely does a fetus-newborn have purely symmetric or asymmetric IUGR; more commonly, the condition would be considered "mixed," particularly at gestational ages of <34 weeks.[3] How many newborns were close to that asignment?

4. Risk factors for adverse fetal outcome were identified for comparison. Many are not independent factors, such as young age and pregnancy-induced hypertension. Other factors are poorly correlated with IUGR (e.g., urinary tract infection, anemia, maternal age >35 years) and still others could be more completely defined (poor obstetric history, antepartum hemorrhage, poor weight gain). More refined groupings might unmask significant correlations.

5. These high-risk factors were compared by χ^2 analysis with little significance identified. However, it would have been useful to evaluate the difference between the population of IUGR patients and the general data base to confirm that this population was indeed unique.

6. Perinatal events and fetal outcome would benefit from more detailed assessment. For example, the single category "intrapartum fetal distress" includes meconium-stained amniotic fluid, abnormal fetal heart rate patterns, and fetal acidosis. These three clinical findings may be independent and may or may not reflect "distress" or fetal compromise.

Differences in 5-minute Apgar score and neonatal death rate were statistically significant between the symmetric and asymmetric groups. However, the symmetric IUGR newborns were more likely premature. Is this association simply a reflection of prematurity?

In summary, Dr. Lin and colleagues have presented interesting material from a large perinatal data base. With their patient selection no antepartum maternal or fetal factors clearly differentiated symmetric from asymmetric IUGR. I look forward to additional analysis of this information.

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DR. L. WAYNE HESS, Jackson, Mississippi. Dr. Lin's article leaves several questions unanswered, including the following: (1) What percent of cases of antenatally suspected IUGR did not have IUGR postnatally (per Dr. Lin's definition)? (2) Was there a difference between the outcomes of the 227 antenatally diagnosed and 491 postnatally diagnosed IUGR infants; i.e., did antenatal intervention improve outcome? (3) How was the control group defined for Fig. 2? (4) What was the power of the statistics for Tables I to IV to conclude there were no statistical differences?

Additionally, the study could be strengthened by the addition of a case-matched control group. A recent study contrasted all IUGR infants with a case-matched control population.[1] However, this study failed to contrast type I and type II IUGR infants to the control population. To date, no other large series has contrasted type I and type II IUGR infants to a control population.

Dr. Lin's article provides the clinician some new insights into the problem of IUGR. However, it leaves the clinician with three problems that are difficult to reconcile. First, the clinician is interested in abnormal growth, not birth weight. A birth weight at the 10th percentile will not always define abnormal fetal growth.[2] A postterm fetus that is severely malnourished will frequently have a birth weight >10th percentile. Second, previous studies that have "established" the 10th percentile for birth weight have demonstrated great variability in results, mainly on the basis of population differences. Lubchenco et al.[3] established neonatal growth curves on the basis of 5635 Denver (high-altitude) hospital births occurring between 1948 and 1961. The "dates" in this study were based on mother's last menstrual period without correction for clinical criteria. Nonwhite and diabetic patients were excluded from the study. Goldenberg et al.[4] noted a 192 gm (at 28 weeks) to 823 gm (at 42 weeks) difference in the 10th percentile birth weights in 38 summarized studies. Third, defining IUGR as a birth weight <10th percentile assumes: (1) that adverse outcome and birth weight percentile remain constant at all gestational ages and (2) that the 10th percentile is a meaningful, discrete cutoff level. Myers and Ferguson[5] have demonstrated that neither of these assumptions is true. The birth weight percentile at which the stillbirth rate is quadrupled is the 1.5th percentile at 28 weeks versus the 16.5th percentile at 42 weeks. IUGR must be more objectively defined before standard obstetric interventions or newly suggested therapies (such as fetal substrate augmentation with nutrients and oxygen or low-dose maternal aspirin therapy) can be objectively, scientifically evaluated.

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DR. DANIEL A. RIGHTMIRE, Springfield, Illinois. I am quite curious as to why Dr. Lin did not use a head circumference/abdominal circumference ratio in his definition of symmetric versus asymmetric growth retardation. Head circumference, compared to abdominal circumference, may be a late indicator of poor fetal growth.

Think of the causes of growth retardation as being divided into placental factors, maternal factors, and fetal factors. I am afraid that Dr. Lin's definition may have preselected a group of small-to-be fetuses who had fetal factors early on, either pathologically or normally. In other words, he may have a mix of constitutionally small babies who will do well and babies with birth defects or other constitutional factors, all of whom may be predetermined to be small throughout pregnancy.

I am afraid that many of his findings might be simple, logical extensions of his definitions rather than reflections of pathologic conditions of gestation.

TRANSACTIONS OF THE FIFTY-SEVENTH ANNUAL MEETING OF THE PACIFIC COAST OBSTETRICAL AND GYNECOLOGICAL SOCIETY

The issue of animal rights and human rights

Presidential address

James M. Maharry
Whittier, California

Those of you who are involved in biomedical research are well aware that animal rights activists have mounted a concerted effort to deny the use of animals in research endeavors. Biomedical research efforts are under attack in the press and electronic media. There have been acts of violence in laboratories. Efforts to obtain research animals and the funds necessary to carry out studies have been compromised by intense lobbying efforts in the U.S. Congress and in the universities.

Two years ago after being named president-elect of this meeting, I selected "animal rights" as the topic for the presidential address. This presented a small problem. I, like most clinicians, knew almost nothing about the subject. To acquire the knowledge needed to address the topic, a simple pseudoscientific study was designed and carried out. The material and methods used, results obtained, and comments will constitute my presidential address.

First, however, the bias of the investigator should be firmly established. To do this, a short autobiography is presented. I was born and reared on a farm in Iowa, where an early Sunday morning ritual was for the children to catch several chickens for my mother to kill for Sunday dinner, a meal prepared and served when the family returned from church in the early afternoon. One of my earliest and most vivid memories occurred on such a Sunday morning. After catching and handing a chicken to my mother, I watched as she wrung its neck. After the neck was wrung and with the head still in my mother's hand, the chicken's body flew through the air to land upright and run across the barnyard for 10 or 15 feet before it collapsed. I stood transfixed until I heard my mother's voice: "Jimmy! For heaven's sake, go pick it up."

My parents had a firm belief in God, and their daily conduct reflected biblical teaching. There was no doubt in their minds that God had given Adam and Eve dominion over all the animals. Along with this right of dominion, they felt a deep moral obligation to humanely care for their animals. Our farm animals were fed, watered, and sheltered from the elements. If ill or injured beyond human help, they were humanely destroyed.

We always had a watchdog that helped with the livestock. He was never allowed in the house, and he subsisted on table scraps and what food he could forage. Off limits to him were chickens and the barn cats. Cats lived in our barn and were expected to sustain themselves by ridding the barn and grainery of rodents. Rodents were not trapped or poisoned. If they could avoid the cats, they were allowed to live. In the 1930s when the government was paying farmers to destroy and bury their livestock, my father refused to join the program. "It was against the nature of things," he said.

We hunted pheasant and ducks in season and rabbits the year-round. When not hunting we fished. Hunting and fishing were not for the sake of the kill but for putting food on the table. We were taught the reverence for life and the interdependence of one life form on another form of life.

My experience in animal research has been limited to the classroom laboratory. In undergraduate school, cadavers of dogfish and domestic cats were dissected in the study of comparative anatomy. In medical school, I learned to pith a frog and euthanize a rabbit by the intravenous injection of a bolus of air. On looking back, I studied remarkably few living animals; most of my time was spent trying to properly smoke a kymograph drum. The medical school I attended did not use live dogs for teaching students; however, live dogs were used in the research laboratory.

I have not hunted or fished since leaving the farm. On acquiring our first home, we adopted the first of our many pet dogs. Our pet dogs have lived in the house as members of the family and forage for food only as far as their bowl on the patio. I must confess

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to occasionally trapping a gopher in the garden, and I once hired an exterminator in a futile attempt to rid our avocado trees of rats.

Material and methods

The true meaning of this scientific heading has been corrupted. For this study it describes the four methods used to obtain material necessary to educate the investigator.

Method 1. Small monetary donations were made to two animal rights groups.

Method 2. An office employee was assigned the duty of spending several hours each week in the morgue of the *Whittier Daily News*, our local newspaper. Her duty was to make copies of press accounts concerning animal rights published since Jan. 1, 1980.

Method 3. Each new issue of the *TV Guide* magazine was studied, and a list of programs to be aired in the ensuing week pertaining to animal rights was prepared. It was my duty to view these at a later time on videotape. Reprints of programs of special interest were obtained for more intense study.

Method 4. Med-Line computer program through Presbyterian Intercommunity Hospital was requested to make a title search of the scientific literature for articles concerning the animal rights issue. Reprints of 30 scientific articles pertaining to this subject were then obtained. A copy of *The Biomedical Investigator's Handbook for Researchers Using Animal Models* was obtained from the Foundation for Biomedical Research.

These four simple methods produced a great volume of material for study.

Results

The small donations to several animal rights groups evidently resulted in the placing of my name and address on a mailing list of people sympathetic to animals, inasmuch as in a very short time six to 10 letters a month from a number of different groups began arriving in the mail. These mailings from animal rights groups, which always contained a plea for funds, can be loosely divided into two types. The first type almost always contains a graphic picture, often of a living dog that appears to have been cruelly mutilated. Another of this type pictures a wild animal held in the jaws of a steel trap. The second type of letter presents a long litany of horror stories of animal abuse. It then goes on to point out cases of animal cruelty they have exposed and their successes in bringing charges against those responsible.

A plea for funds illustrative of the first type of letter was received from the Doris Day Animal League. The package of material contained a four-page letter stating that 10 million innocent animals died horribly last year in needless experiments. It then graphically described the scene of a family pet, Skip, a beagle dog, being led to his death in an animal research facility. Skip was described as lovable, faithful, and as trusting as any family pet that ever lived. The letter leads one to believe that 10 million dogs a year are so cruelly destroyed. The package of material also contained a sealed envelope with this warning: "This envelope contains photographic evidence of the brutal mistreatment of a helpless animal. Do not open if strong, graphic material is offensive to you." The sealed envelope contained the picture of a living dog that had been cruelly mutilated. The package also contained petitions that needed only my signature to forward to my congressman and both California senators. The petitions urged the passage of a federal law to outlaw the use of pound animals in biomedical research.

The second type of plea for funds was contained in material received from the Animal Legal Defense Fund. The envelope that contained their solicitation for funds appeared similar to those received from the U.S. government. Emblazoned on the envelope was: "National Animal Rights Survey." The four-page letter informed me of my being selected to participate in a survey on animal rights. If I could answer "yes" to any of the eight questions in the survey I should send money to support their efforts to protect animals everywhere from exploitation and cruel abuse. The letter implored me to join the fight against the vast research industry that thrived on animal cruelty, noting that each year the billion-dollar industry needlessly "snuffed out" the lives of more than 50 million animals. They further noted that much of the research was funded by my tax dollars, whereas my donation to them was tax deductible. Their unique role in the animal rights movement was emphasized: "Only the Animal Legal Defense Fund has the expertise to deliver legal services that can be crucial to saving an animal's life." The letter also recounted their court room victories for animal welfare on the farm, in the wilderness, and in research laboratories.

Our research conducted in the morgue of the local newspaper produced numerous press reports concerning animal rights activity. Almost all the articles reported acts of violence against universities, biomedical research facilities, U.S. companies, and even department stores. Acts of violence or other attempts at intimidation were reported to have occurred locally, regionally, nationally, and internationally.

Local. There were frequent reports of animal rights activists gathering at Loma Linda University to protest the use of baboons to supply hearts as transplants for human infants.

Statewide. Almost $5 million of damage was caused when militant activists set fire to the veterinarian diagnostic laboratory that was under construction at the University of California at Davis. Terrorist activity intended to intimidate research at the University of California at Berkley was treated by the press as a humorous event. This is the case in which pickets mounted cranes to stop construction of a research building on the campus but chose the wrong building site.

Animal rights activists are not only challenging the right to use animals in biomedical research, agricultural scientists and producers of livestock, eggs, chickens, and milk are also under increasing attack. In Dixon, California, the last sheep auction yard in the state was shut down by a $250,000 arson fire. Earth First, a radical environmental group, claimed responsibility for the fire; they also took credit for vandalism to the California Cattlemen's office in Sacramento.

Regional. The research laboratory at the University of Oregon was damaged to the extent of $40,000, and 125 research animals were stolen. In Tucson, Arizona, a group known as the Animal Liberation Front claimed responsibility for a $100,000 arson fire at the University of Arizona. Not only were research buildings torched, but more than 1000 animals were freed. Among the animals freed were 30 young mice infected with *Cryptosporidium*, an infectious third-world disease.

National. The first act of terrorism noted in our review occurred in 1981 in Rockville, Maryland, where radical animal rights activists burglarized an animal research laboratory and removed four baby chimpanzees worth $100,000. Police in Norwalk, Connecticut, arrested a female terrorist outside of the United States Surgical Company—a company that uses dogs in the research of suture material. She was charged with attempted murder and possession of explosive devices. The head injury laboratory at the University of Pennsylvania was forced to shut down after videotapes of experiments were stolen in a burglary and made available to the public and to Congress.

International. In England, the Animal Liberation Front claimed responsibility for firebomb attacks on five of London's leading department stores. These attacks were made during the Christmas shopping season and caused millions of dollars in damage. The reason for the attacks was that the stores sold coats made from animal furs.

It is disturbing that only one press report defended the use of animals in medical research. This was found in Ann Landers' column in her answer to a reader's letter condemning animal research. She quoted Dr. David Hubel of Harvard Medical School as stating that whereas medical researchers are using other methods such as computers and cell cultures, it just is not true that there is no longer a need for animals. Hubel, who studies abnormalities in the brain that lead to blindness, uses animals and computers. He points out that unfortunately there is nothing in a computer that resembles a brain and that any research about the brain that involves a computer is based on earlier studies with animals. She also quoted Dr. Donald Kennedy, president of Stanford University, as stating that important research cannot be done today without animals. The harsh truth is that to stop research with animals would be to stop critically needed fundamental research in biology and medicine. She further quoted Dr. Michael DeBakey, who performed the first successful coronary artery bypass, who stated virtually every pioneering development in cardiovascular surgery was based on animal experimentation. In this field, as in a number of others, there is virtually no alternative to the use of animals for experimental advances.

A 1989 poll conducted by the Gallup Company reported 77% of Americans approved the use of animals for research but only 30% knew the Federal Government regulates the treatment of these animals.

The study of programs broadcast on television that concern animal rights revealed the subject was discussed primarily on talk shows. The spokesman for animal rights would be an impassioned activist detailing atrocities inflicted on helpless animals, quoting wild unsubstantiated figures as to the number of animals cruelly and needlessly destroyed, and stating that had cadavers or cultures and computer models been used, better results would have been obtained. The thalidomide tragedy was often cited to support this argument. One of the effective techniques used by animal rights groups is for their spokesman to be a celebrity who has instant recognition. In contrast, spokesmen for the biomedical research industry are professional in their manner but often appear uncomfortable. Their defense of the use of animals is always the same—without animal research none of us would enjoy the health benefits we have today.

After watching the ABC News *Nightline* television program broadcast on April 25, 1989, a reprint of the program was obtained. The three panelists on the program were a spokesman from the American Medical Association, the leader of People for the Ethical Treatment of Animals, and Jane Goodall. In the introduction to the program it was stated that there were 7000 animal rights groups with 10 million members. The spokesman for the biomedical research industry was low-key and professional, whereas the spokesman from People for the Ethical Treatment of Animals was impassioned and strident in his comments. The moderator had to frequently warn him not to interrupt the other panelists. People for the Ethical Treatment of Animals took credit for using the stolen videotapes to shut down the head injury laboratory at the University of Pennsylvania.

Jane Goodall's contribution was poignant and obviously very sincere. She acknowledged that her mother's life had been saved by a heart valve obtained from a pig. She mourns the pigs that gave their lives in research and those that would continue to lose their lives to improve human existence. Her closing remarks were that she looked forward to the day when there would be no animals held in cages.

The review of the scientific literature was undertaken only to educate this investigator. Here is a short list of some titles[1-18] representative of the type of articles being published in the scientific literature concerning animal experimentation: *The First 100 Years of Vivisection: 1824 to 1924*,[1] *William Osler and Arthur Conan Doyle Versus the Antivivisectionists*,[2] *Immoral and Moral Uses of Animals*,[3] *Science and Animal Protection*,[4] *Regulation of Animal Experimentation*,[5] *The Cost of Not Doing Medical Research*,[6] *The Case for the Use of Animals in Biomedical Research*,[7] *The Physician's Stake in Animal Research*,[8] *Rethinking the Morality of Animal Research*,[9] *The Biomedical Investigator's Handbook for Researchers Using Animal Models*,[10] *The Use of Animals in Biomedical Research*, the AMA "white paper," 1989.[11]

Comment

Historically, animal experimentation has been carried out in a very limited manner since ancient times. It only began in earnest, however, early in the nineteenth century.[1, 2] From its inception, people with concern for animals began to take notice, and in 1823 the Royal Society for the Prevention of Cruelty to Animals was founded in England. By the middle of the nineteenth century, opposition to the use of animals was well organized. This organized opposition was labeled antivivisectionism. At about this time in France, Claude Bernard, the founder of physiology, was opposed in his research efforts by his wife and daughter, who were antivivisectionist leaders. The first national law relating to animal experimentation was enacted in England in 1876. The Society for the Prevention of Cruelty to Animals was founded in the United States in 1866, and the first bill opposing animal experimentation was introduced in the U.S. Congress in 1897. The present law (Animal Welfare Act) governing animal experimentation in the United States was enacted in 1966, and last amended in 1985. This act applies primarily to cats, dogs, and subhuman primates.

This brief and incomplete history is presented only to make you aware of the long history of organized opposition to animal experimentation. Traditionally, humane societies were formed to prevent the inhumane treatment of animals everywhere, whereas antivivisectionists, now known as animal rights activists, have concerned themselves with animal experimentation.

In the past 10 years many new and militant animal rights groups have been formed, and militant activists are infiltrating long-established humane societies. In taking over humane societies long-established endowments will be available to them.

Over the past 10 years, the rhetoric of animal rights activitists has become increasingly violent. This violent rhetoric is stimulating those on the lunatic fringe of society to commit the acts of terrorism that are increasingly reported. It has been said violence in the voice is often only the death rattle of reason in the throat. On television talk shows, panelists and some of the audience applaud acts of violence committed by militant activists. Violence in the support of any cause is an act of terrorism. Those responsible for burglary, arson, or any other criminal act should be prosecuted, not applauded, for their illegal behavior.

This is not intended to be a blanket condemnation of all animal rights groups or their well-meaning members. For more than 150 years the organizations with genuine concern for the welfare of animals have served as the conscience of society. The old cliche that if you eat them or wear them you have no moral right to oppose their use in biomedical research should never be used in the defense of animal experimentation. That argument is simplistic, undignified, and simply not true. The vast majority of the members who peacefully support these groups with their time and money have every moral right to do so.

The danger in all of this is the recent and rapid evolution of a huge animal rights industry, an industry probably far larger in numbers than the biomedical research industry. Opportunists are forming new groups and others are infiltrating established ones. In an effort to attract new members and new donations, they are using the most modern techniques. These techniques include mailing lists, bulk mailings, public relations firms, and fund-raising organizations. I recently watched my first national television commercial presented by People for the Ethical Treatment of Animals. The animal rights industry is big business with the so-called cruelly exploited and defenseless animal as the centerpiece. There is a concerted effort by the Animal Legal Defense Fund to be named as the legal surrogate for animals. If they are successful they can sue in court for monetary damages on behalf of animals.

Unfortunately for society, no one in organized medicine or in biomedical research seems willing to able to oppose such efforts. If you examine the scientific literature on the subject of animal rights you are overwhelmed by its sheer volume. On reading this literature one is immediately impressed by the depth of soul searching evident in the text of many of the articles. Unfortunately, there is a fatal flaw contained in this vast literature concerning animal rights—researchers and members of the medical profession are just talking to each other. A perfect example of this fact is occurring here today. After almost 2 years of study, I am speaking to a collection of 200 people, most of whom are already sympathetic to animal experimentation. If, by chance, these remarks are published, it will be in a journal primarily read by those who practice obstetrics and gynecology. Few will read it.

The biomedical research industry and the medical profession may be under a siege more grave than even the industry itself realizes. Translating the percentages from the Gallup Poll[17] into population figures reveals: 60 million Americans do not approve the use of living animals in research, and 150 million know almost nothing about the subject. This may portend an ominous future for society.

Something must be done about the fact that in our scientific literature we are only talking to each other. Unfortunately, we only listen when the subject directly concerns us. Why shouldn't we adopt the same modern techniques being used against us? There is nothing evil or unethical about using mailing lists, bulk mailings, public relations experts, or even fund-raising organizations in the name of education. Effective speakers should be recruited from the medical profession to carry our case to the public. The biomedical research industry has genuine concern for animals used in necessary experimentation; *The Biomedical Investigator's Handbook*[10] and the vast literature on the subject amply demonstrate this fact. Researchers use in vitro rather than in vivo methods whenever possible; three of the four largest cosmetics companies have just abandoned the Draize test and have gone to an in vitro test.

The American public is a reasonable body and would accept a truthful recounting of the prodigious advances in biological and medical knowledge that have resulted from the necessary use of animals in biomedical research. The public needs to realize *there is not a single modern and substantial item of medical knowledge that does not owe something to animal experimentation.* The health and welfare of animals has improved markedly as a result of experimentation.

When a movie or television personality has a successful by-pass surgery, we should not hesitate to let society know of the role animals played in the development of the surgical procedure. Professional athletes who have insulin-dependent diabetes are praised for their courage. Without animal experimentation there would be no insulin to control the disease. Parents of young children need to know of the role animals played in the development of the vaccines that protect all of society from recurrent epidemics. The public needs to be informed of the role animals play in the safety of the medications they use in their daily lives. After development and release to the public, all drugs and biologicals are subjected to continued testing on animals to ensure their safety; this testing is mandated by federal law. Companies that produce chemicals used in household products are mandated by law to certify their safety. This requires that large numbers of animals be killed to carry out the lethal dose 50 test. Cosmetics companies must certify their products to be safe for human use; this testing, again with the use of animals, is mandated by federal law.

The number of animals killed each year in biomedical research and product testing can only be estimated, but whatever the exact number, at least 90% are rats and mice. Dogs make up less than 2% of the total. The fact that 15 million household pets are killed each year in the name of animal control by the local animal pound elicits only a feeble public outcry.

At times one has to research the so-called comic strips to discover pointed social commentary. The following is a dialog between Sir Rodney and the King in a strip from the *Wizard of Id.* Sir Rodney addresses the King who is seated on this throne, "Sir! You have to do something for the homeless!" The King answers, "Where am I going to get the money?" Sir Rodney replies, "Could we cut back a bit on the new animal shelter?" The priorities of our society are such that the picture of an injured animal elicits a stronger humanitarian response than do pictures of the homeless huddling on the streets or tiny children dying of starvation in Ethiopia.

I respect the moral right of a person to protest the use of animals in biomedical research or product testing, but how much better the world would be if their efforts and money were directed toward making shelter available for the homeless, food available to the hungry, a guarantee of safety for all drugs and products used by animals and human beings, and a cure for all the diseases that plague both animals and man.

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Lymphocytic adenohypophysitis: A pituitary mass lesion occurring in pregnancy

Proposal for medical treatment

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Lymphocytic adenohypophysitis is a nonneoplastic, autoimmune cause of pituitary enlargement and insufficiency. Forty-eight of the 50 reported cases have occurred in women, nearly all in association with pregnancy. Left undiagnosed and untreated, it can progress to pituitary insufficiency and death. Histologic studies show characteristic changes of autoimmune disease with lymphocytic infiltration and destruction of anterior pituitary tissue with fibrotic replacement. Lymphocytic adenohypophysitis is currently diagnosed after other pituitary mass lesions are excluded and has been treated with a combination of neurosurgery and end-organ hormone replacement. However, with improved knowledge of the pathophysiologic characteristics and natural history of the disease and with the ability to make a prospective diagnosis, we believe glucocorticoids may suppress the inflammatory response and protect remaining pituitary tissue. Two previously unreported pregnancy-associated cases are described, including one prospectively diagnosed and treated without neurosurgery during pregnancy. Obstetrician-gynecologists must place lymphocytic adenohypophysitis in the differential diagnosis of pituitary enlargement associated with pregnancy, since treatment is available and the sequelae may be life-threatening. (AM J OBSTET GYNECOL 1991;164:1549-55.)

Key words: Lymphocytic adenohypophysitis, pituitary, pregnancy complications, neurosurgery, pituitary insufficiency

Lympyhocytic adenohypophysitis is a nonneoplastic, autoimmune cause of pituitary enlargement and insufficiency. Of 50 reported cases in the world literature, 48 have occurred in women, and nearly all have occurred during pregnancy, the puerperium, or the first postpartum year. Despite this association with pregnancy, there is rare mention of this disease in the obstetric and gynecologic literature. If left undiagnosed and untreated, it can progress insidiously or quickly to pituitary insufficiency and death. Obstetrician-gynecologists and neurosurgeons must therefore include lymphocytic adenohypophysitis in the differential diagnosis of pregnancy-associated pituitary enlargement. Often mistakenly diagnosed as a prolactin-secreting adenoma, the etiology, pathology and therapy of the two differ fundamentally. Lymphocytic adenohypophysitis is currently treated with a combination of neurosurgery and end-organ hormone replacement, often resulting in significant chronic morbidity, whereas prolactin-secreting adenomas may be more easily managed medically and usually without long-term sequelae. With improved knowledge of the pathophysiology of lymphocytic adenohypophysitis, the ability to make a prospective diagnosis among symptomatic peripartal women, and recently acquired experience in treatment, we believe that a trial of medical therapy may be warranted before routine neurosurgical exploration is performed. We report here our experience with two previously unreported cases of this unusual cause of pituitary enlargement in young peripartal women including the first case prospectively diagnosed and treated without neurosurgery during pregnancy.

Case reports

Case 1. A 26-year-old primiparous woman was seen, 1 month after uncomplicated vaginal delivery, with a 2-month history of daily midline frontal headaches accompanied by diplopia and gradual decrease in visual acuity. During the postpartum period, she had failure of lactation and complained of lethargy and low-grade fever. Her medical history, including gynecologic, was noncontributory. Physical examination revealed bitemporal field defects to confrontation, left greater than right, with acuity of 20/60 on the left and 20/40 on

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the right. A computed tomographic (CT) scan showed a large pituitary mass with suprasellar extension. Laboratory evaluation demonstrated hypopituitarism; insulin-induced hypoglycemia resulted in a basal cortisol level of <1 μg/dl with no increase after stimulation and a growth hormone level of 3.1 ng/ml with no increase. Thyrotropin-releasing hormone challenge (200 μg given as an intravenous bolus) resulted in a prolactin increase from 4 to 8 ng/ml; a gonadotropin-releasing hormone challenge (100 μg) was followed by a luteinizing hormone rise from 4 to 10 mIU/ml, a follicle-stimulating hormone rise from 9 to 20 mIU/ml; and decreased thyroid function (thyroxine index, 0.9; thyroxine, 3.7 μg/dl; triiodothyronine, 23.5 ng/dl). An autoantibody screen was negative, including antinuclear antibodies, latex fixation, antithyroglobulin, and antimicrosomal antibodies. The hypopituitarism was attributed to either a pituitary adenoma or adenohypophysitis. With corticosteroid coverage, transsphenoidal pituitary exploration was performed. A large, dull, white, tough mass extending almost 2 cm above the sella was subtotally removed. Histologic examination showed infiltration and destruction of anterior hypophyseal acini with lymphocytes and plasma cells (Fig. 1, *A*) and replacement of normal glandular tissue with lymphoid aggregates and fibrosis (Fig. 1, *B*). After operation the patient's visual acuity and fields returned to normal. She was discharged and is being followed up on a regimen of hormone replacement including hydrocortisone, desmopressin acetate, levothyroxine, conjugated estrogens, and medroxyprogesterone acetate.

Case 2. A 30-year-old multiparous woman was admitted to the hospital in her thirty-fifth week of gestation with a complaint of blurred vision. Her antenatal course had been uncomplicated until about 6 weeks before admission, when she noted gradual onset of blurred vision in the left eye that progressively worsened; at admission she was found to have bitemporal hemianopsia. Menses had been regular before conception, and there was no history of infertility or galactorrhea. Pregnancy studies, including an ultrasonogram and nonstress tests, were normal. A CT scan showed a large intrasellar mass with suprasellar extension (Fig. 2, *A*). Laboratory analysis included normal complete blood count and SMA-12 results for pregnancy, and a prolactin level of 185 ng/ml (within normal limits for 35 weeks' gestation). An overnight adrenocorticotropic hormone stimulation test was performed. Basal cortisol level was 9 μg/dl with a rise to 13 μg/dl. Dynamic pituitary testing was postponed until after delivery. Tests for rheumatoid factor, antinuclear, anti-double-stranded dexoyribonucleic acid, antimitochondrial, anti–smooth muscle, anti–parietal cell, antimicrosomal, and antithyroglobulin antibodies were negative. Serologic test for syphilis was negative. The differential diagnosis was lymphocytic adenohypophysitis versus pituitary adenoma. Bromocriptine therapy (2.5 mg twice daily) was started as a diagnostic maneuver and to reduce pressure on the optic chiasm. Amniocentesis was performed; the lecithin/sphingomyelin ratio was 1.8 : 1 and no phosphatidylglycerol was present. Daily visual field studies were performed and remained unchanged. After 9 days of bromocriptine therapy there was no improvement in symptoms, and a repeat CT scan was obtained that showed no change in the size of the mass. Prednisone therapy (2.5 mg three times a day) was instituted. Nine days later, after completion of 37 weeks' gestation, the patient was delivered of a 3360 gm infant after an uncomplicated induction of labor. She was maintained on a regimen of prednisone. During the first postpartum week her clinical condition and visual fields remained unchanged, and she lactated normally without breastfeeding. One week after delivery, vision began to improve and subsequently returned to normal. A CT scan performed at that time revealed a decrease in the size of the mass, although significant enlargement persisted. Menses resumed 3 months post partum without breastfeeding. CT scan performed at that time showed a significant decrease in mass size (Fig. 2, *B*). Dynamic pituitary testing was performed 5 months post partum, after tapering and temporarily stopping steroid therapy for 6 weeks before testing; insulin-induced hypoglycemia showed undetectable growth hormone before and after stimulation and a cortisol level of 4 ng/dl with no rise, a thyrotropin-releasing hormone challenge showed a rise in prolactin level from 79 to 357 ng/ml, and a gonadotropin-releasing hormone challenge resulted in a luteinizing hormone rise from 10 to 91 mIU/ml and a follicle-stimulating hormone rise from 7 to 25 mIU/ml. She has been maintained on a regimen of prednisone and is having regular menses. Human leukocyte antigen haplotypes were A28, Aw24(A9), Bw44(B12), B7, Cw7, Dr2, Dr5, MT1, MT2, and MT4.

Comment

Management of uncommon diseases that occur during pregnancy has been enhanced by better prospective diagnosis and knowledge of the pathology and natural history of these conditions. Enlargement of the pituitary gland during pregnancy is one such area in which recent anatomic and laboratory studies have contributed to more specific diagnosis and, for some pituitary mass lesions (e.g., prolactin-secreting adenomas), a change from primarily neurosurgical management to medical treatment. In a young pregnant woman with a large expanding pituitary lesion, the most common diagnosis remains a prolactin-secreting adenoma. However, the differential diagnosis must also include lymphocytic adenohypophysitis. The importance of making this diagnosis prospectively is underscored by the natural history of the disease, which has resulted in death in 25% of patients reported with this disease, including the first five in whom it was recognized at autopsy. In a recent review of 35 English language cases, individual patient presentations were summarized.[1]

Presentation. Lymphocytic adenohypophysitis is a

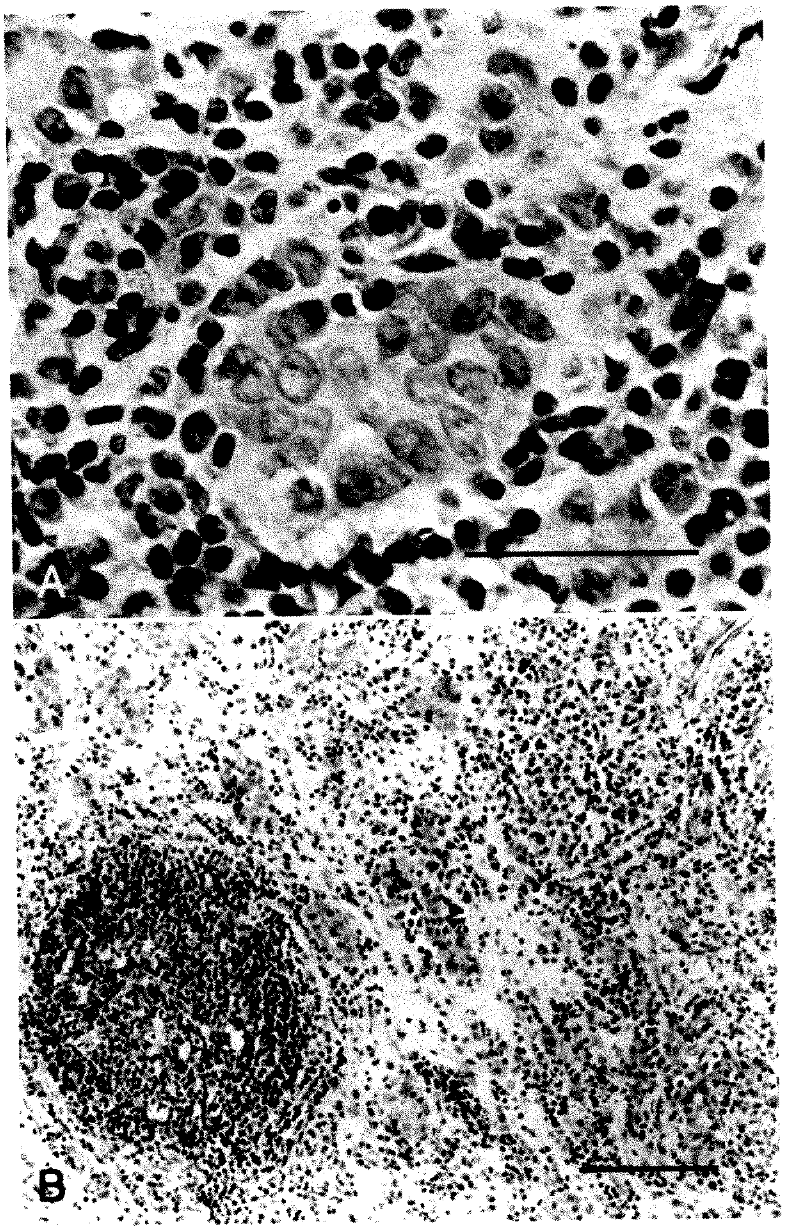

Fig. 1. A, Pituitary acinus surrounded by lymphocytes and plasma cells. (Hematoxylin and eosin. Original magnification ×343; *bar* = 100 μm.) **B,** Lymphoid aggregates in the anterior pituitary with destruction of normal tissue and replacement by fibrosis. (Hematoxylin and eosin. Original magnification ×205; *bar* = 100 μm.)

disease predominantly of women (96% of all reported cases). The youngest reported case occurred in a 17-year-old,[2] and the oldest in a 69-year-old; the median reported age at diagnosis was 28 years. Both men[3, 4] were diagnosed in their fifth decade of life. Lymphocytic adenohypophysitis is reported most often in association with pregnancy; 65% of reported cases were multiparous women and 35% had disease associated with a first pregnancy. But it has been reported in women who have never been pregnant (6% of cases). The majority of pregnant patients present with symptoms during the last trimester, although it has been reported in all trimesters. No racial predilection has been detected. The presentation appears to correlate with the apparent stage of the disease process at time of diagnosis. In nearly every case in which diagnosis is made in a living patient, presenting complaints are referable to mass effects of headache and visual changes, symptoms caused by the inflammatory infiltrate, and resulting edema of the pituitary gland. When the condition is not diagnosed antemortem, most patients have had progression to lethargy, anorexia, hypoglycemia,

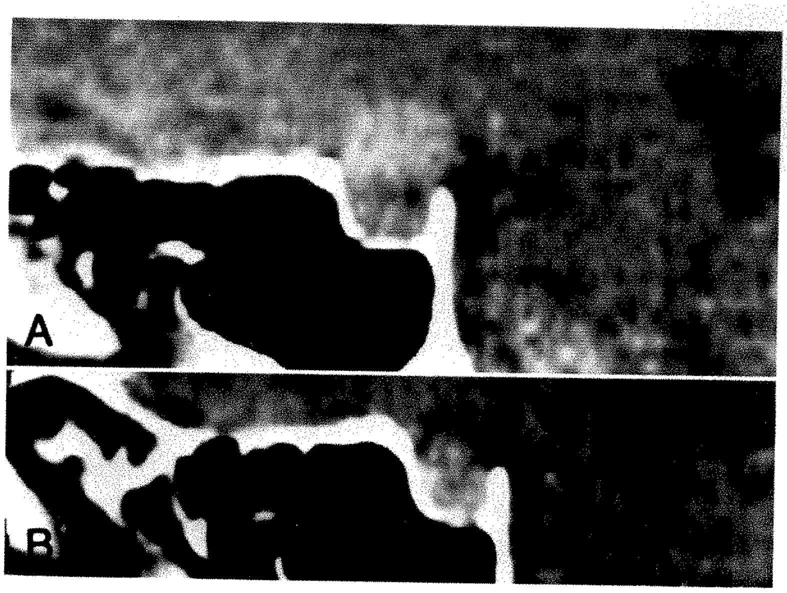

Fig. 2. A, CT scan through sella turcica of case 2 at initial presentation, 35 weeks' gestation. **B,** Case 2 at 3 months post partum with corticosteroid therapy. Significant decrease in size of mass is evident compared with 2, **A.**

and frank secondary adrenal insufficiency as a result of acute or chronic hypopituitarism. Most recent reported cases have had at presentation symptoms of a space-occupying sellar lesion, i.e., visual disturbances with or without headaches, rather than symptoms associated with adrenal insufficiency. This was the case for the four patients treated at this institution, including two previously reported.[5] In spite of the presenting symptoms referable to cranial compression, 75% of reported peripartal cases of lymphocytic adenohypophysitis had some evidence of partial or panhypopituitarism at some time during pregnancy or the postpartal period, although endocrine evaluation was incomplete in many. Among pregnant patients with sufficient reported information, hypoadrenalism (78%) and thyroid dysfunction (72%) were the most frequent abnormalities found. Primary thyroiditis diagnosed at autopsy or by clinical and laboratory criteria was found in six patients. Some patients may have only isolated defects of a single pituitary factor, e.g., adrenocorticotropic hormone. Measurement of gonadotropins and prolactin is not useful for making the diagnosis of lymphocytic adenohypophysitis during pregnancy but may be helpful in nonlactating (seven cases) and amenorrheic (nine cases) women after an interval following delivery. Only two cases of posterior pituitary dysfunction before neurosurgical intervention have been reported.[6, 7]

Anatomy and pathology. Lymphocytic infiltration of the anterior pituitary occurs rarely. Shanklin[8] examined 100 unselected human pituitaries and found a 43% incidence of lymphocytic infiltration, but this was confined entirely to the pars intermedia and pars nervosa with no involvement of the anterior lobe. Simonds and Brandes[9] reported the presence of lymphocytes in the anterior pituitary of two of 200 patients with sudden death. These series are in marked contrast to a recent report[10] describing mild lymphocytic adenohypophysitis in five of 69 autopsy-obtained pituitaries from women who died during pregnancy, after abortion, or during the postpartum period and emphasizing the association of lymphocytic adenohypophysitis with pregnancy. However, it was not clear how the lymphocytic adenohypophysitis related to each patient's illness.

Enlargement of the pituitary gland is a normal component of the anatomic and physiologic changes that occur during pregnancy[11] and is due principally to hyperplasia of prolactin-secreting cells,[10] but such enlargement does not normally induce symptoms of a space-occupying cranial lesion. Our cases were diagnosed with CT technology. In 27 of 30 patients (90%) having CT scans, a sellar lesion was demonstrated at the time of initial scan. Use of polytomography (eight abnormal in eight cases) and plain skull radiography (seven abnormal in 14 cases) has also been reported, and magnetic resonance imaging has been used in the evaluation or follow-up of six patients with lymphocytic adenohypophysitis.[1, 12] In nearly all abnormal radiologic studies of lymphocytic adenohypophysitis, a suprasellar component was demonstrated, regardless of the imaging technique used. Radiologic features have been reviewed in detail by Levine et al.[13] and Miura et al.[7]

The operative specimens obtained by transsphenoidal resection at our institution were similar to those described in the literature, i.e., firm or tough, dull white-yellow, and adherent to the surrounding structures. Histologic examination showed lymphoid infiltration, diffuse and in aggregates, and fibrosis that nearly completely obliterated normal pituitary architecture. No abnormal cells or oncocytic transformation was recognized. Numerous reports have demonstrated the presence of all major T-lymphocyte subpopulations, as well as plasma cells, in these lesions, by means of immunohistochemical staining techniques specific for surface markers and presence of κ–light chains. Review of reported postmortem cases supports the observation that the degree of replacement of normal glandular tissue with lymphocytic infiltrate is correlated with the stage of the disease process at the time of death. Those patients in whom sudden death was preceded by fatigue, anorexia, confusion, weakness, and shock most often had an atrophic gland at autopsy. An enlarged infiltrated pituitary gland also may be found in conjunction with atrophic adrenal glands.

Association with autoimmunity. The nature of the histologic findings and the natural history of lymphocytic adenohypophysitis suggest an autoimmune etiology. The histologic characteristics seen in the pituitary are similar to those seen in other autoimmune diseases, i.e., lymphocytic infiltration, obliteration of glandular tissue, and replacement of normal tissue with fibrosis. Many patients also have autoimmune-mediated inflammatory involvement of other organs, including thyroiditis,[2, 14] pancreatitis, parathyroiditis, pernicious anemia, adrenalitis, and connective tissue inflammation. In one patient[12] sarcoidosis of the eye and lung developed 6 months after diagnosis of lymphocytic adenohypophysitis associated with the birth of her second child. Too few human leukocyte antigen haplotypes have been reported to reveal specific genetic associations, although Bw35, found in increased frequency in autoimmune disorders, was reported in two of four cases when tissue typing was performed.

One classification scheme[15] defines a process as an autoimmune disease if it fulfills three criteria: (1) detection of circulating autoantibodies to a recognized specific antigen, (2) the production of similar pathologic changes in an experimental animal model produced by injection of antigen, and (3) transfer of the disease by immune serum or cells. In general, patients with lymphocytic adenohypophysitis have had negative general antibody screens, but most have not been screened for antipituitary activity. Antipituitary antibodies have been difficult to demonstrate in patients with lymphocytic adenohypophysitis in whom these studies have been attempted. Difficulties have resulted from the small amount of material obtained from operation, low apparent titers of specific antibodies, and nonspecific binding. Specific antipituitary antibodies have been found in three of six patients tested, but antibodies were not found in patients in other reports. Autoantibodies to the pituitary also have been reported in 23 of 128 unselected postpartum women in one study.[16] In that study, in 25% of 16 antibody-positive postpartum women available for follow-up, some symptoms of pituitary insufficiency developed. Experimental animal models of lymphocytic adenohypophysitis have been described. Levine[17] produced adenohypophysitis in adult rats with injection of adjuvant and pituitary lymphocytic infiltrate. From preliminary experiments, he suggested that the severity of the process was increased in postpartum animals.

Differential diagnosis. Lymphocytic adenohypophysitis must be considered in the differential diagnosis of women in the peripartum period with symptoms consistent with a space-occupying cranial lesion. In addition to sella imaging and visual field evaluation, laboratory studies including electrolytes, thyroid function tests, and a basal and adrenocorticotropic hormone–stimulated cortisol should be obtained promptly during pregnancy. A CT scan yields better information for prospective management compared with conventional radiologic studies, although the radiation exposure is somewhat higher. Magnetic resonance imaging poses no known risk to the fetus or mother and, if available, may be expected to yield information helpful in distinguishing lymphocytic adenohypophysitis from other pituitary masses. Bromocriptine (2.5 mg twice daily) should be started immediately. Initial laboratory results, sella imaging, and response to bromocriptine should suggest the direction of further evaluation and the need for prompt initiation of corticosteroid and other hormone replacement.

Postpartum women with failure of lactation and those whose menses fail to resume post partum should be closely questioned and examined for other features referable to pituitary insufficiency. In addition to sella imaging and visual field testing, an insulin-induced hypoglycemia test should be performed and growth hormone, prolactin, and cortisol concentrations assessed. A gonadotropin-releasing hormone and thyrotropin-releasing hormone challenge can be performed the same day. Since thyrotoxicosis is an occasional concomitant finding both during the peripartal period and among patients with lymphocytic adenohypophysitis, thyroid function studies should be performed and blood should be held for antibody studies.

Pituitary insufficiency may be seen in other groups of peripartum patients. Patients with postpartum pituitary necrosis, i.e., Sheehan's syndrome, can demonstrate a similar pituitary stimulation test profile. However, among lymphocytic adenohypophysitis patients diagnosed in or after labor and delivery, uncomplicated vaginal deliveries have been the rule, except for one patient in whom the disease was not suspected and who died in labor of acute adrenal insufficiency.[1] Other cases of pituitary insufficiency in association with pregnancy have been described, usually with imaging consistent with a partially empty or empty sella. Increased secretion of prolactin has been described in patients with lymphocytic adenohypophysitis and an enlarged or normal-sized[18] pituitary. This may lead the unwary to diagnose a prolactin-secreting adenoma and institute bromocriptine therapy without further investigating other pituitary trophic hormone secretion. Glycoprotein-producing pituitary adenomas, also known as nonsecretory or null-cell adenomas, may present as mass lesions, also unresponsive to bromocriptine. These adenomas may exhibit decreased pituitary trophic hormone secretion, most commonly decreased gonadotropins, and mildly elevated prolactin as a consequence of stalk compression. Increased prolactin secretion may be expected during and some weeks after pregnancy and is insufficient by itself to diagnosis a pregnancy-associated pituitary mass. Endocrinologically active pituitary tumors, e.g., growth hormone-producing tumors, usually exhibit some degree of isolated pituitary hyperfunction in association with pituitary enlargement.

Treatment. Transsphenoidal chiasmal decompression with initiation of preoperative and postoperative hormone replacement is the traditional diagnostic and therapeutic approach. However, there is reason to believe that, at least for some patients, there may be a less radical initial approach. Most patients reported in the recent literature have undergone only partial surgical resection or pituitary biopsy in the absence of marked chiasmal compression. The main benefit derived from this potentially morbid and expensive procedure may be establishment of the histologic diagnosis. However, consistent clinical features, supportive laboratory findings, and nonresponse to bromocriptine may allow a presumptive, noninvasive diagnosis of lymphocytic adenohypophysitis.

Since lymphocytic adenohypophysitis appears to share pathophysiologic features common to many autoimmune disorders, we believe that aggressive initiation of corticosteroid therapy may induce remission, as well as protect remaining pituitary tissue during periods of acute involvement. Some experience with corticosteroid therapy has been obtained. One 18-year-old woman, 10 weeks postpartum, was found to have secondary adrenal insufficiency, postpartum thyrotoxicosis, hypogonadism, and a pituitary mass with suprasellar extension.[19] Surgery was deferred due to thyrotoxicosis and because there were no symptoms referable to chiasmal compression. She was treated with cortisone acetate and propranolol hydrochloride and experienced nearly complete resolution of the mass. She continued to have chronic partial pituitary insufficiency of ACTH, growth hormone, thyroid-stimulating hormone, and prolactin production and remains on hormone replacement. At least five patients have been treated with corticosteroids in anticipation of surgical resection with improvement in signs or symptoms.[1] One patient[20] was followed after being placed on steroids when she presented in the thirty-ninth week of gestation with visual field defects. Similar to our case 2, she experienced resolution of her mass but had residual pituitary insufficiency. Another patient,[21] not operated upon, had gradual recovery of thyroid function, but not of ACTH, prolactin, or growth hormone after being placed on corticosteroids post partum.

Specific management should be determined by response to bromocriptine and steroids, as assessed by changes in visual fields, changes in mass size on imaging studies, and recovery or progression of endocrine deficits. Whether patients will be followed prospectively or undergo resection, with evidence of pituitary insufficiency of ACTH, one should not delay in instituting corticosteroids while exploring other etiologies or organ involvement. One of the two men reported with lymphocytic adenohypophysitis developed a typical adrenal crisis resulting in death while undergoing diagnostic workup for panhypopituitarism, despite being placed on corticosteroids early in the crisis.[3] Two patients gradually regained normal pituitary function after transsphenoidal decompression,[1] one patient regained normal thyroid function only,[22] and in one postpartum patient pituitary insufficiency gradually developed where none had existed before operation.[23] One suspected antepartum case of lymphocytic adenohypophysitis with selective deficiency of prolactin and growth hormone[24] continued to be deficient post partum although a pituitary mass regressed. Another patient in whom the disease was suspected, with symptoms of a pituitary mass but no hormone deficits during pregnancy, did not undergo operation. Post partum she experienced regression of the mass but later pituitary insufficiency of adrenocorticotropic hormone, growth hormone, and prolactin developed.[25] These cases underscore the rationale for continued observation and retesting of these patients. Two patients with lymphocytic adenohypophysitis have achieved a subsequent pregnancy, as would be expected with appropriate hormone replacement.

With time, acute pregnancy-associated maternal immune system alterations resolve after delivery and may enable withdrawal of steroid therapy with careful assessment of residual function. Such patients should be monitored closely for evidence of chronic or acute pituitary insufficiency and for occurrence of features associated with other autoimmune disorders. Corticosteroid therapy may not obviate the need for neurosurgical intervention in some cases of pregnancy-associated lymphocytic adenohypophysitis, but it is hoped that surgery may be reserved for patients with acute chiasmal compressive lesions that are nonresponsive to either bromocriptine or corticosteroids.

In summary, lymphocytic adenohypophysitis must be included in the differential diagnosis of pregnancy-associated pituitary enlargement. If it is diagnosed, a trial of medical therapy with corticosteroids may allow conservation of remaining pituitary tissue and decrease morbidity and costs associated with caring for patients with this unusual pituitary mass lesion.

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Antibiotic treatment of tuboovarian abscess: Comparison of broad-spectrum β-lactam agents versus clindamycin-containing regimens

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One hundred nineteen patients with tuboovarian abscess were evaluated for response to antibiotics. Results were stratified into three groups by antimicrobial regimen. Group 1 consisted of 37 patients treated with a single-agent broad-spectrum intravenous antibiotic and oral doxycycline. Initial clinical response (defined as decreased pain, diminished white blood cell count, or defervescence) in group 1 was 31/37 (84%). Group 2 consisted of 64 patients treated with clindamycin in combination with an aminoglycoside with or without a penicillin. There was an initial clinical response in 45 of 64 (70%). Group 3 consisted of 18 patients from group 1 who were changed to a clindamycin-containing regimen after 2 to 3 days of initial treatment with a single-agent broad-spectrum antibiotic. The decision to switch antibiotics was not based on treatment failure but occurred when delayed ultrasonography confirmed the diagnosis of tuboovarian abscess. The switch reflected physician preference for clindamycin-containing regimens in the treatment of tuboovarian abscesses. The response rate in this subset of patients was 14 of 18 (78%). Overall initial clinical response rate was 90 of 119 (75%). There were no statistically significant demographic or clinical differences among the three groups. There was no statistical difference in the rate of early and late antibiotic failure rates among the groups. Our study demonstrates that extended-spectrum antibiotic coverage, including single-agent broad-spectrum antibiotics such as cefoxitin, in conjunction with doxycycline has efficacy that is equivalent to that of clindamycin-containing regimens. An overall medical treatment success rate of 75% suggests that conservative treatment of tuboovarian abscesses is warranted. (AM J OBSTET GYNECOL 1991;164:1556-62.)

Key words: Tuboovarian abscess, pelvic abscess, pelvic inflammatory disease, tuboovarian complex, antimicrobial therapy

Tuboovarian abscesses have long been recognized as a severe form of pelvic inflammatory disease that typically results from inadequate or delayed treatment of a pelvic infection. Tuboovarian abscesses are reported in 3% to 16% of hospitalized patients with acute pelvic inflammatory disease.[1] Management of tuboovarian abscesses has evolved markedly from the purely surgical approach of the preantibiotic era, which included extraperitoneal drainage with an associated high mortality from ruptured tuboovarian abscesses. When antibiotics were introduced, aggressive intraabdominal surgical approaches were used to treat ruptured tuboovarian abscesses; also antibiotics were used in the treatment of unruptured tuboovarian abscesses to "cool a patient off" in preparation for delayed surgery.[2] We are now evolving toward a more conservative approach, in which potent and broad-spectrum antibiotics are used as definitive first-line therapy and in which surgical intervention occurs only in tuboovarian abscesses that do not respond to initial antibiotic therapy.

Research on the medical treatment of tuboovarian abscess indicates that proper antimicrobial treatment of pelvic abscesses should include antibiotics that penetrate abscess cavities and remain stable in the abscess environment.[3] In particular, antimicrobial agents that provide adequate coverage against the resistant gram-negative anaerobes of *Bacteroides* group should be used. Antibiotic regimens containing clindamycin or metronidazole have generally been considered the best available agents for the treatment of tuboovarian abscesses.[1, 5] In 1983 we reported data on 321 patients with tuboovarian abscesses, only 10 of whom had been treated with one of the now commonly used extended-spectrum second- and third-generation cephalosporins.[1] The patients in that study were treated with either a clindamycin-containing regimen or a regimen with inadequate anaerobic coverage. The overall initial clinical response rate with antibiotic treatment alone was

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70%, and the response rate for those treated with clindamycin (89%) was significantly better than that of patients who received non-clindamycin-containing regimens (35%). The current study compares antimicrobial regimens containing a single-agent broad-spectrum antibiotic that is effective against the resistant gram-negative anaerobes with clindamycin-containing regimens. The predominant regimens used were cefoxitin plus doxycycline and clindamycin plus gentamicin.

Methods

One hundred twenty-five patients were discharged from San Francisco General Hospital between April 1980 and January 1989 with a diagnosis of tuboovarian abscess (palpable adnexal or pelvic mass confirmed by ultrasonography or computed tomography). Six patients underwent surgical treatment of tuboovarian abscess within 48 hours of admission without an antibiotic trial and thus were excluded. The charts of the remaining 119 patients were retrospectively reviewed. Patients had been enrolled previously in treatment trials of acute pelvic inflammatory disease that were approved by the University of California, San Francisco, Committee on Human Research.

In all 119 patients either an initial clinical diagnosis of tuboovarian abscess was confirmed by ultrasonography with the exception of two patients who had computed tomography scan confirmation or ultrasonography after admission demonstrated a tuboovarian abscess in instances in which one had not been clinically appreciated. The ultrasonographic diagnosis of tuboovarian abscess required demonstration of a well-defined complex adnexal mass ≥4 cm in size with no apparently normal ipsilateral ovary. In the patients who required surgical intervention, all clinically and ultrasonographically diagnosed tuboovarian abscesses were confirmed at surgery. All masses were at least 4 cm in diameter and were complex in nature by imaging techniques.

Cultures were obtained in the majority of patients. Endocervical, endometrial, and abscess specimens were evaluated for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. In addition, the endometrium and abscess were sampled for anaerobic and aerobic bacteria.

Cultures of the cervix for *N. gonorrhoeae* were obtained with a Dacron swab, inoculated directly onto modified Thayer-Martin agar plates, and held at 35° C in 5% carbon dioxide. Cervical specimens for *C. trachomatis* were collected with a sterile calcium alginate–tipped swab that was placed immediately in minimal essential media containing antibiotics and 10% fetal calf serum and refrigerated at 4° C until transport to the laboratory for isolation. Most specimens were processed within a few hours of acquisition, and all were processed within 24 hours.

A double-lumen, plastic telescoping catheter containing a wire brush and sealed with a gelatin plug was used for collection of the endometrial specimens. Endometrial brushes were divided into two parts under sterile conditions. One was processed for *C. trachomatis*, and the other for isolation of *N. gonorrhoeae* and other aerobic and anaerobic bacteria.

All specimens for *C. trachomatis* were inoculated onto monolayers of cycloheximide-treated McCoy cells by centrifugation. The cells were cultured for 65 hours, then examined for inclusions by either iodine or immunofluorescent staining. One blind passage was performed on all negative specimens.

Specimens were processed for isolation of facultative organisms by inoculation onto the following media, which were incubated with 5% carbon dioxide at 35° C: trypticase soy agar with 6% sheep blood, chocolate agar, calcium nutrient agar with 6% human blood, modified Thayer-Martin agar, and calcium nutrient agar with 6% sheep blood. Specimens processed for anaerobic growth were inoculated into *Brucella* agar supplemented with 6% sheep blood, vitamin K, and hemin, calcium nutrient agar with 6% sheep blood, kanamycin-vancomycin laked blood agar, and chopped meat carbohydrate broth incubated at 35° C in either a Gas-Pak jar (BBL, Cockeysville, Md.) or an anaerobic chamber (Coy Manufacturing, Ann Arbor).

N. gonorrhoeae and facultative bacteria were identified by standard methods. Anaerobic organisms were identified with gas-liquid chromatography and prereduced, anaerobically sterilized media.

Patients were placed in three groups according to antimicrobial treatment regimen (Table I). Group 1 consisted of 37 patients treated with a single-agent extended-spectrum antibiotic combined with doxycycline in most instances. Cefoxitin plus doxycycline (n = 27) was the most common regimen. Patients in group 2 (n = 64) were treated with clindamycin, usually in conjunction with an aminoglycoside with or without a penicillin. Patients in group 3 (n = 18) were initially treated with a group 1 regimen for 2 to 3 days and then were switched to a group 2 regimen. The decision to change antimicrobial agents was not due to treatment failure but rather was based on physician bias toward clindamycin-containing regimens when delayed ultrasonography revealed the presence of a tuboovarian abscess. The groups were compared with respect to clinical presentation, initial response to antibiotics, and treatment failure. Initial response was defined as defervescence, decreased pain, decrease in mass size, and discharge from hospital without need for surgical intervention. Early treatment failure was defined as lack of an initial clinical response plus institution of surgical intervention or a switch in antimicrobial therapy or both.

Table I. Antibiotic regimens used for treatment of tuboovarian abscesses

Group 1 (n = 37)	*Group 2 (n = 64)*	*Group 3 (n = 18)*
Cefoxitin and doxycycline (*n* = 27)	Clindamycin and tobramycin (*n* = 37)	Cefoxitin and doxycycline; clindamycin and aminoglycoside (*n* = 15)
Cefotetan and doxycycline (*n* = 5)	Clindamycin and gentamicin (*n* = 24)	Cefotetan and doxycycline; clindamycin and aminoglycoside (*n* = 2)
Ampicillin and sulbactam (*n* = 2)	Clindamycin and kanamycin (*n* = 1)	Ampicillin and sulbactam; clindamycin and aminoglycoside (*n* = 1)
SCH 34343 and doxycycline (*n* = 1)	Clindamycin and aztreonam (*n* = 1)	
Ciprofloxacillin (*n* = 2)	Clindamycin and ciprofloxacillin (*n* = 1)	

Table II. Clinical variables in patients with tuboovarian abscesses

	Group 1 (*n* = 37)	*Group 2* (*n* = 64)	*Group 3* (*n* = 18)	*Group 4* (*N* = 119)
Mean age (yr)	27.3 ± 7.4	28.0 ± 8.3	30.3 ± 7.2	27.7 ± 7.9
Parity	1.1 ± 1.3	0.85 ± 1.1	0.94 ± 1.1	0.98 ± 1.2
History of PID	20 (55.0%)	28 (43.7%)	7 (41.1%)	55 (46.0%)
Mean temperature (C)	38.3° ± 0.66°	38.2° ± 0.6°	38.6° ± 0.4°	38.3° ± 0.65°
Mean WBC ($\times 10^6$/ml)	14.6 ± 4.2	15.4 ± 11.7	16.3 ± 5.0	15.0 ± 9.0
Mean ESR (mm/hr)	63.5 ± 27.1	65.0 ± 28.9	66.5 ± 30.4	64.3 ± 28.0
Mean mass size (cm)	7.2 ± 2.2	6.7 ± 2.5	7.4 ± 2.0	6.9 ± 2.4
Days of intravenous antibiotics	7.8 ± 2.2	7.2 ± 2.3	9.4 ± 2.6	7.5 ± 2.3

PID, Pelvic inflammatory disease; *WBC,* white blood cell count; *ESR,* erythrocyte sedimentation rate.

Follow-up after discharge from hospital consisted of return visits at 2 weeks, with some follow-ups continuing >1 year after discharge. Clinical response at follow-up was defined as diminished or absent pelvic mass by pelvic examination or ultrasonography and diminished or absent pain. Late treatment failure was defined as a persistent mass or pain at follow-up. Seventy-five patients returned for follow-up.

Descriptive statistical analysis was used to compare the groups. Discrete variables were analyzed standard χ^2 test. Continuous variables were analyzed with the unpaired *t* test. A p value of <0.05 was considered statistically significant.

Results

There were no statistical differences among the three groups with regard to age, parity, and history of pelvic inflammatory disease (Table II). All patients were premenopausal and were aged 14 to 50, with a median of 27. The mean duration of hospital stay was 7.5 days, with a median stay of 7 days. There was no statistically significant difference in demographic characteristics among the groups (Table II).

Clinical data analysis revealed a mean temperature of 38.3° ± 0.6° C, a mean white blood cell count of 15.0 ± 9.0 × 10^6/ml and a mean sedimentation rate of 64.3 ± 28 mm/hr. There were no statistically significant differences among the treatment groups with regard to clinical presentation (Table II). Endometrial and cervical cultures done on admission demonstrated pathogens similar to those in our previously reported series.[4] Only 90 patients had both endocervical and upper genital tract microbiologic specimens obtained. *N. gonorrhoeae* was recovered from the endocervix in 49 patients (54.4%), and *C. trachomatis* was recovered in six patients (7%). From the endometrial cavity *N. gonorrhoeae* was recovered in 36 patients (40%), *C. trachomatis* from 6 patients (7%), and mixed anaerobic and aerobic bacteria from 68 patients (75%). The most common group of microorganisms recovered from the patients with tuboovarian abscesses included mixed anaerobic and facultative bacteria, predominantly *Bacteroides* sp., *Bacteroides bivius, Peptostreptococcus* sp., and *Gardnerella vaginalis.* Neither *C. trachomatis* nor *N. gonorrhoeae* were recovered from aspirates of the tuboovarian abscesses. There was no statistical difference with regard to pathogens among the groups.

Initial clinical response, defined as diminished pain, white blood cell count, or temperature, is summarized in Table III. In group 1, 31 of 37 patients (84%) responded to antimicrobial treatment and were discharged from the hospital without surgical intervention. An initial clinical response was observed in 45 of 64 patients (70%) in group 2 and in 14 of 18 patients (78%) in group 3. These differences were not statistically significant.

Overall, 90 of 119 patients (75%) initially responded to antibiotics alone. The remaining 29 (25%) under-

Table III. Initial clinical response to antibiotic therapy

	Successful response (N = 119) (No.)
Group 1	31/37 (84%)
Group 2	45/64 (70%)
Group 3	14/18 (78%)
TOTAL	90/119 (75%)

went surgical intervention because of early treatment failure. Of these, 17 (59%) underwent total abdominal hysterectomy with bilateral or unilateral salpingo-oophorectomy. Nine patients (31%) had unilateral adnexectomy and three (10%) had colpotomy drainage. All clinically and ultrasonographically diagnosed tuboovarian abscesses were confirmed in the 29 patients who underwent operation (Table IV). An additional 11 patients had late failure. Five patients underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy >2 months after initial treatment. The remaining six patients had continued pelvic pain and masses but refused surgical intervention. The long-term clinical response rate with initial antibiotic therapy alone was 79 of 199 (66.5%).

Seventy-five patients underwent follow-up after discharge (Table V). Twenty-seven of 28 patients (96.4%) in group 1 had a diminished or absent pelvic mass on physical examination or follow-up ultrasonography. Twenty-five of 34 patients (73.5%) in group 2 and 12 of 13 patients (96.2%) in group 3 showed a clinical response at follow-up. On χ^2 analysis there was a statistically significant increase in follow-up response in group 1 patients compared with those in group 2 ($p = 0.036$). There were no statistical differences between groups 1 and 2 and groups 2 and 3.

Analysis of the influence of mass size on treatment response rates was similar among all three groups. In general, the trend indicated that the larger the mass, the greater the likelihood that the patient would not respond to antibiotics and would need surgery. This relationship increased dramatically in mass sizes >10 cm (Fig. 1).

Long-range follow-up (>1 year) was difficult to obtain in these patients. Of the patients available for long-term follow-up, 6 of 75 achieved an intrauterine pregnancy (4 in group 1, 2 in group 2, and 0 in group 3). The pregnancy rate of 8% is an underestimate because whether the patients were actively seeking pregnancy was not ascertained.

Comment

Management of acute unruptured tuboovarian abscesses remains controversial. Several authors have advocated prompt aggressive surgical treatment to decrease long-term morbidity and mortality.[6, 7] More recently, favorable results have been reported with conservative therapy consisting of broad-spectrum antibiotic treatment in an attempt to preserve reproductive potential.[1, 8, 9] Our study supports a conservative approach with an overall initial clinical response rate of approximately 75%. Only 25% of our patients needed surgical intervention after an adequate trial of broad-spectrum intravenous antibiotics during initial hospitalization. Most patients have an initial response within 72 hours of initially receiving antibiotics.[1] Over the long term an additional 11 patients (9.2%) were considered failures; this resulted in an overall success rate of 65.8%.

Numerous studies have now documented successful response rates in patients with tuboovarian abscess who are treated conservatively with antibiotics. However, there is a lack of data comparing the various regimens, particularly the newer second- and third-generation cephalosporins. Previous reports have suggested that improved response rates are achieved by using agents that penetrate abscesses and that are active against the resistant gram-negative anaerobes, such as *B. fragilis* and *B. bivius*. Cefoxitin and many of the third-generation cephalosporins and extended-spectrum penicillins fall into this category. In 1985 Hemsell et al.[10] reported a 95% initial success rate in using cefotaxime to treat 41 patients with tuboovarian abscesses. They did not, however, compare these results with a clindamycin- or metronidazole-containing regimen. In this study we found that cefoxitin in combination with doxycycline compared favorably with clindamycin-containing regimens in the treatment of tuboovarian abscesses. Group 3 reflected our preconceived notion that clindamycin was superior to cefoxitin and therefore that patients who were not immediately responding or who were discovered later to have a tuboovarian abscess were generally switched to a clindamycin-containing regimen. There were no striking differences whether clindamycin was added (group 3) or was not added (group 1), and the results of both groups compared favorably with those of the clindamycin-containing regimens. This study supports the efficacy of cefoxitin and similar advanced-spectrum cephalosporins in the treatment of tuboovarian abscesses. Changing antibiotic regimens in patients with pelvic inflammatory disease to a clindamycin- or metroidazole-containing regimen because a tuboovarian abscess is diagnosed does not appear to alter the outcome in the majority of cases.

Surgical intervention was required during the initial hospitalization in 29 of our patients (25%). A conservative approach with unilateral adnexectomy or colpotomy drainage was used in 11 (40%). None required

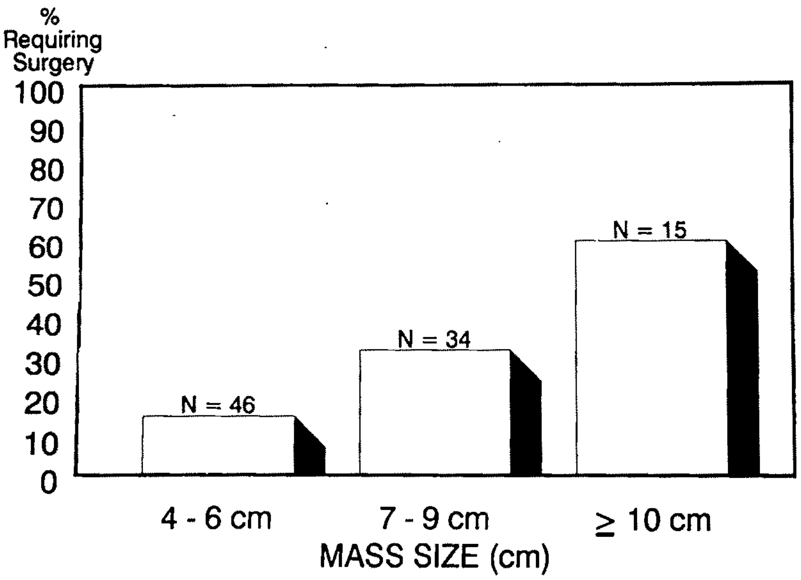

Fig. 1. Relationship between size of tuboovarian abscess and need for surgical intervention. An additional two patients had surgery recommended but left without further follow-up.

Table IV. Surgical treatment: Early antibiotic failure

	Group 1 (*n* = *37*)	*Group 2* (*n* = *64*)	*Group 3* (*n* = *18*)	*Overall*
Adnexectomy	1	7	1	9
TAH ± BSO, USO	4	11	2	17
Colpotomy	1	1	1	3
TOTAL	6 (16%)	19 (30%)	4 (22%)	29 (25%)

TAH, Total abdominal hysterectomy; *BSO*, bilateral salpingo-oophorectomy; *USO*, unilateral salpingo-oophorectomy.

Table V. Follow-up response

	Long-term response (*n* = *75*) (*No.*)
Group 1	27/28 (96.4%)
Group 2	25/34 (73.5%)
Group 3	12/13 (96.2%)

subsequent hysterectomy. Rivlin and Hunt[11] initially advocated this approach in 1977. In their report 57 patients in a series of 113 (50%) with tuboovarian abscess rupture underwent bilateral adnexal procedures and postoperative peritoneal lavage. Thus 50% of patients had management consisting of conservative surgical procedures.

This study emphasizes the importance of using imaging techniques to improve the quality of care in the management of tuboovarian abscesses. All patients included in the study had a mass ≥4 cm confirmed either ultrasonographically (98.3%) or by computed tomography scan (1.7%). All patients with a mass <4 cm determined either ultrasonographically or by physical examination were excluded from the study as a result of the difficulty in distinguishing a pyosalpinx from a tuboovarian abscess. Most important, all of these patients responded to medical management and did not need surgery. Fig. 1 shows the relationship between mass size and the success of medical therapy. These results emphasize the importance of mass size as determined by pelvic examination or ultrasonography or both. Follow-up of tuboovarian abscesses for increasing size also may indicate antibiotic treatment failure.

More conservative surgical treatments with laparoscopic drainage or unilateral adnexectomy or both have been advocated. Henry-Suchet et al.[12] reported 50 cases managed by laparoscopically directed drainage in 1984. Ninety percent (45 patients) had "complete initial clinical recovery"; 44 of the 45 had long-term follow-up. Twenty percent of those patients subsequently underwent total abdominal hysterectomy–bilateral salpingo-oophorectomy or unilateral adnexectomy. Thirteen percent had chronic pelvic pain, and 19% had bilateral tubal occlusion at follow-up laparoscopy. Twenty-eight percent had a normal pelvis on follow-up laparoscopy, and 11% had unilateral adnexal damage. Thus, at follow-up, 40% of patients had at least one patent tube.

Reich et al.[13] treated 21 women with tuboovarian abscesses with intravenous antibiotics and laparoscopic drainage. Twenty of the 21 had complete resolution. One underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. Six of the 20 subsequently achieved pregnancies. There were no laparoscopic injuries.

Computed tomography–guided drainage of intraabdominal abscesses is well documented.[14] Only recently have there been reports on computed tomography–guided drainage of tuboovarian abscesses. Worthen and Gunning[15] reported on 33 patients with computed tomography–guided or ultrasonographically guided percutaneous drainage of tuboovarian abscesses after 24 hours of intravenous antibiotics. The overall success rate was 77%. Complications occurred in two patients (6%); one had a bowel laceration and the other had rupture of the tuboovarian abscess. Tyrell et al.[16] had no complications in their series of eight patients. Seven of the eight had failure of conservative medical therapy (3 to 12 days of intravenous ampicillin, gentamicin, and clindamycin with little or no improvement), and one patient underwent emergency computed tomography–guided drainage after 24 hours of triple antibiotic therapy because of her critical condition. Seven of the eight (88%) had initial complete recovery and did not need surgery. Twenty months after treatment, one patient had recurrent symptoms that were presumed to have resulted from a new pelvic infection.

Although these studies of laparoscopic and percutaneous drainage are encouraging, there are still no randomized controlled studies comparing antibiotic treatment alone with antibiotics in combination with a drainage procedure. Moreover, these studies have not addressed the issues of long-term outcome, such as preservation of fertility. Additional studies are needed to prospectively evaluate the efficacy of new antibiotic regimens alone and in combination with laparoscopically or ultrasonographically guided drainage procedures. Until such data are available, conservative antimicrobial therapy is an appropriate initial approach in the management of a suspected tuboovarian abscess. The therapeutic regimen used must include an agent that is effective against β-lactamase–producing anaerobes, penetrates abscesses, and remains active within the abscess environment. As a result of our experience, three fourths of patients with tuboovarian abscess should respond to conservative medical therapy alone.

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Discussion

DR. GLEN E. HAYDEN, Seattle, Washington. Drs. Reed, Landers, and Sweet are to be congratulated for an excellent study of the second- and third-generation antibiotics, especially the cephalosporins. The three major regimens are more formalized than those of the original 321 patients with tuboovarian abscess reported on in 1983.[1] The newer antibiotics meet the qualifications for penetration into the abscess cavity and remain active. Dr. Sweet has demonstrated that the tuboovarian abscesses resulted primarily from mixed anaerobic bacteria. The absence of the common sexually transmitted organisms is remarkable.

The conservative or initial medical approach is well supported by the successful data. The fact that 25% needed surgical intervention is important, but 40% of the surgical procedures were conservative. The clinical success rate of 75% with medical management is exceptional, but the cost factor for ≥7 days of hospitalization and 24-hour antibiotic therapy is staggering. The quoted cost ranges from $6,000 to $8,000; the unit cost to the patient of 6 or 12 hours is the same for all antibiotics. The newer antibiotics offer improved quality of treatment but as yet no reduction of cost.

As Dr. Sweeet has postulated, the ultimate aim of the treatment of tuboovarian abscess is the preservation of fertility and the prevention of pain or recurrent episodes. The need is for long-term randomized controlled studies of antibiotics versus antibiotics with conservative surgery.

There is sufficient evidence to support the idea of early ultrasonographically guided drainage or laparoscopic drainage of abscesses >10 cm. Adequate preantibiotic and postantibiotic therapies are essential. Operative laparoscopy is becoming more inventive with the development of jet stream dissection, irrigation, and suction of the abscess pocket. The three-way stopcock and 5 mm blunt suction device at the surgical level allow for repeated forceful irrigation and suction of an abscess site. The open laparoscopy approach is safe and useful in the more difficult abdomen.

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Dr. Kenneth A. Burry, Portland, Oregon. This article describes the conservative treatment of a very difficult condition. I am interested in infertility and wonder if you have any long-term follow-up of fertility of these patients and the outcomes of their pregnancies.

Dr. Mary Martin, San Francisco, California. Dr. Sweet, please comment on the absence of the detection of *Chlamydia* in these cultures.

Dr. Carl Goetsch, Berkeley, California. I am old enough to have had personal experience in St. Louis with the treatment of this terrible disease without the aid of antibiotics. It was more harrowing than is suggested by the brief description by Dr. Sweet. Those patients took a very long time to arrive at a point where we felt it was safe to operate. We tried to provide supportive therapy until their temperatures had come down and had stabilized at a relatively low level or, as we would say, they had "cooled off." Then we would go in and evacuate the abscess. If it was accessible through the cul-de-sac, we did a colpotomy and put in a drain that we made on the spot from a 3/4-inch rubber tube. We cut it lengthwise and put two holes in it. We reached in with a clamp and pulled the "ears" out so that it made a very satisfactory T drain. If we could not drain the abscess through the cul-de-sac, we usually waited a long time. When we finally operated, even though occasionally the cyst would rupture, the result was very satisfactory. The patient would promptly become afebrile if she had had any low-grade fever. There was practically never any peritonitis, and we never got positive cultures from the purulent-appearing contents. The abscess had "burned out." When antibiotics came along (I was still in my training at that time), things began to look brighter for the treatment of this disease.

Dr. Sweet (Closing). Dr. Hayden, in the article I addressed the relative paucity of sexually transmitted disease organisms in the upper genital tract—the fallopian tubes, the abscesses, and the cul-de-sac. We actually found only one patient with gonococcus and none with chlamydia. I agree that the cost is extraordinary with these patients and would average >$8,000 for a week in the hospital. On the other hand, in our experience, patients who need surgery have 10 or 12 days of hospitalization at a much greater cost and greater risk of infertility. It is a dramatic change to be able to preserve fertility in the management of tuboovarian abscesses.

Dr. Hayden, I agree that we urgently need a randomized, controlled prospective study comparing modern conservative surgery to antibiotics alone versus the traditional surgical approach versus antibiotics alone. I hope that we do not find ourselves 5 or 10 years from now in a situation similar to that which we now have with the question of fetal monitoring, a situation in which it seems that we have an ethical problem to "go back" and do the appropriate study. There is no question that Henry-Suchet et al.,[1] Reich et al.,[2] and others have demonstrated good response with laparoscopic drainage. However, it is difficult to decide whether the cases they reported were all true tuboovarian abscesses.

Dr. Burry, we do have long-term data that I did not have time to present. In this study 8% of the 75 patients that we were able to follow up conceived within 1 year. In our initial study we had a 14% pregnancy rate after conservative medical therapy. This is not a great number, but it is better than zero, which is what you have if you do a hysterectomy and bilateral salpingo-oophorectomy. I think it is also important to recognize that the pregnancy rate is a minimum, because we do not ask the women whether they are trying to become pregnant. We feel it is an underestimate by 50%.

Dr. Martin, the absence of chlamydia in abscesses is consistent with what we have seen for a long time. In uncomplicated pelvic inflammatory disease, our chlamydia isolation rate has been about 15%, with a range from 10% to 25% over the last decade. However, we have never recovered *Chlamydia trachomatis* via monoclonal antibody stains, cultures, or even deoxyribonucleic acid probes, from the purulent material in a tuboovarian abscess. It is not clear whether the chlamydia organism is killed by an overgrowth of anaerobes, which we have postulated may happen with *N. gonorrhoeae*. The damage by Chlamydia is not from a purulent-type infection such as that associated with gonococcus but from the release of inflammatory mediators such as tumor necrosis factor and interferon, which produce tissue damage in the tubal epithelium.

Dr. Goetsch, you have succinctly and eloquently outlined for us the changes in management of tuboovarian abscesses. We have progressed a great deal since the preantibiotic era in preservation of fertility and hormonal function.

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Antepartum testing in the hypertensive patient: When to begin

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Antepartum testing has been recommended for patients whose pregnancies are complicated by hypertension. Although this is considered accepted practice, there are little data available to help the clinician know when to start testing. To help answer this question in patients with chronic hypertension and nonproteinuric pregnancy-induced hypertension, we reviewed the results of all antepartum tests between 1976 and 1987 in patients with these diagnoses. The primary mode of surveillance in the majority of our patients was the contraction stress test. We determined when patients first had positive contraction stress test results and when intervention occurred because of an abnormal antepartum test result. There were a total of 917 patients tested with these diagnoses. Fifty-three (5.8%) of these patients had at least one positive contraction stress test result. Twenty-two patients were delivered of infants before 35 weeks' gestation because of abnormal antepartum test results. Those with early intervention (<35 weeks' gestation) could not be differentiated from those with later intervention (≥35 weeks' gestation) by maternal age, diastolic blood pressure, or systolic blood pressure at the time of testing. The majority of patients who were delivered of infants before 35 weeks' gestation had a concomitant diagnosis of systemic lupus erythematosus, intrauterine growth retardation, diabetes mellitus, or superimposed preeclampsia. On the basis of when compromise was evident, patients with these diagnoses may require testing to be started as early as the fetus is considered viable. However, in those without these diagnoses, the clinician may delay the beginning of testing until 33 weeks' gestation without significant risk of pregnancy loss before testing. (AM J OBSTET GYNECOL 1991;164:1563-70.)

Key words: Antepartum fetal testing, hypertension, pregnancy-induced hypertension

Patients whose pregnancies are complicated by hypertension are at increased risk of intrauterine fetal death. In an attempt to prevent such an outcome, antepartum fetal surveillance has been advocated. Although it is accepted practice to initiate antepartum testing in these patients, there are limited data to suggest when testing should begin. Empirically, recent reviews have suggested starting testing anytime from 32 to 36 weeks' gestation unless the disease is poorly controlled or there is evidence of intrauterine growth retardation (IUGR).[1-3] In these clinical situations, it has been recommended that testing be started earlier.

In an attempt to better answer this question, we reviewed all antepartum fetal deaths and all antepartum fetal test results in pregnant patients with a diagnosis of chronic hypertension or nonproteinuric pregnancy-induced hypertension at the initiation of testing. Chronic hypertension was defined as hypertension diagnosed before pregnancy. Nonproteinuric pregnancy-induced hypertension was defined as hypertension first diagnosed during the studied pregnancy. With these data we will attempt to devise a scheme for timing the initiation of fetal testing in this patient population.

Material and methods

All patients tested at Memorial Womens Hospital, Long Beach, California, between January 1976 and December 1987 with a diagnosis of hypertension were evaluated for entrance in the study. Those with an isolated diagnosis of proteinuric pregnancy-induced hypertension (preeclampsia) were excluded from the study because it is generally accepted that these patients are at risk of uteroplacental insufficiency and should have testing started as soon as the disease process is apparent.[4] Patient history, antepartum fetal test results and pregnancy outcome were prospectively collected and retrospectively reviewed.

Recorded patient history included maternal age, gravidity, parity, gestational age when tested, testing method, result of test, systolic and diastolic blood pressures at the time of testing, gestational age at delivery, indication for delivery, other medical diagnoses, and birth weight. Small for gestational age (SGA) was defined as a neonate with a birth weight less than the 10th percentile for a given gestational age using California growth charts.[5] For purposes of this study, IUGR in-

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6/6/29211

Table I. Stillbirths in patients with chronic hypertension or nonproteinuric pregnancy-induced hypertension

Patient	*Age (yr)*	*EGA at IUFD (wk)*	*Test*	*IUGR*	*Complications*
1	35	21	ND	No	Diabetes mellitus
2	32	26	ND	No	Diabetes mellitus
3	26	33	NST	Yes	Superimposed preeclampsia
4	25	34	ND	No	Renal disease
5	29	35	ND	Yes	Superimposed preeclampsia
6	33	37	ND	No	Diabetes mellitus, substance abuse, superimposed preeclampsia
7	32	37	ND	Yes	None
8	26	40	ND	Yes	Superimposed preeclampsia
9	29	40	ND	No	Superimposed preeclampsia
10	16	40	ND	No	Diabetes mellitus

ND, Not done; *NST*, nonstress test; *EGA*, estimated gestational age; *IUFD*, intrauterine fetal death.

cluded both those patients delivered of an SGA neonate and those patients who clinically demonstrated antepartum lag in fetal growth.

In the majority of patients, the primary mode of surveillance was a weekly contraction stress test. Testing procedures and test interpretation are described elsewhere.[6] Patients with multiple equivocal contraction stress test results or a contraindication to stress testing were followed with nonstress tests at frequent intervals. Tests were considered reactive if there were two accelerations that reached a peak of 15 beats per minute above the baseline and lasted 15 seconds in a 20-minute window of recording time. If the nonstress test result was nonreactive or if there was evidence of significant fetal heart rate deceleration on nonstress test, a contraction stress test or biophysical profile was performed. Biophysical profiles were performed and interpreted as outlined by Manning et al.[7] except for interpretation of amniotic fluid volume. In this study, the minimal acceptable amniotic fluid volume was a 2 cm vertical pocket free of extremities and umbilical cord. Intervention because of an abnormal test result was dependent on the specific clinical situation and was the decision of the primary care physician often in consultation with the perinatal staff. Indications for intervention were prospectively recorded. All test interpretations were performed and recorded prospectively. Each contraction stress test and nonstress test was read by at least two perinatal staff physicians.

If the antepartum fetal test is to be effective, it must begin before significant fetal compromise has begun. For this reason, the following three occurrences were used to represent evidence of fetal compromise: (1) gestational age at intrauterine fetal death; (2) gestational age at the first positive contraction stress test result; and (3) gestational age when intervention occurred because of abnormal antepartum fetal test result.

Patients with such evidence of compromise were compared to those without such evidence for maternal age, gravidity, parity, other associated medical illnesses, systolic and diastolic blood pressure levels at the time of testing, and birth weight. Comparisons were made using the Student t test, one-tail Fisher exact test, χ^2 with Yates correction. All variances expressed are plus or minus 1 SD.

Results

Antepartum fetal tests were performed on 8189 high-risk obstetric patients during the years studied. Of those tested, 408 had a diagnosis of chronic hypertension and 509 had a diagnosis of nonproteinuric pregnancy-induced hypertension at the initiation of testing. The mean maternal age, gravidity, and parity were 29.1, 2.6, and 0.9, respectively.

During the study period there were 10 stillbirths that occurred among all hypertensive patients admitted to Women's Hospital. A list of these cases is shown in Table I. It should be noted that nine of the 10 patients were not among the antepartum fetal test study population because they presented with fetal deaths and never had fetal testing. The earliest stillbirth occurred at 21 weeks' gestation. This patient had a concomitant diagnosis of diabetes mellitus. The next earlier stillbirth also occurred in a patient with diabetes who was 26 weeks' gestation at the time of death. It is of interest that both of these patients were delivered of fetuses with true knots in the umbilical cord. Five of the stillbirths occurred in patients with superimposed preeclampsia. None of the fetuses had congenital anomalies. Only one patient had testing initiated before the intrauterine death, and that patient's last test was a reactive nonstress test fewer than 7 days before the fetal death.

There were five neonatal deaths among the 917 patients tested (Table II). Three of them were attributed to respiratory distress syndrome, one of which also had

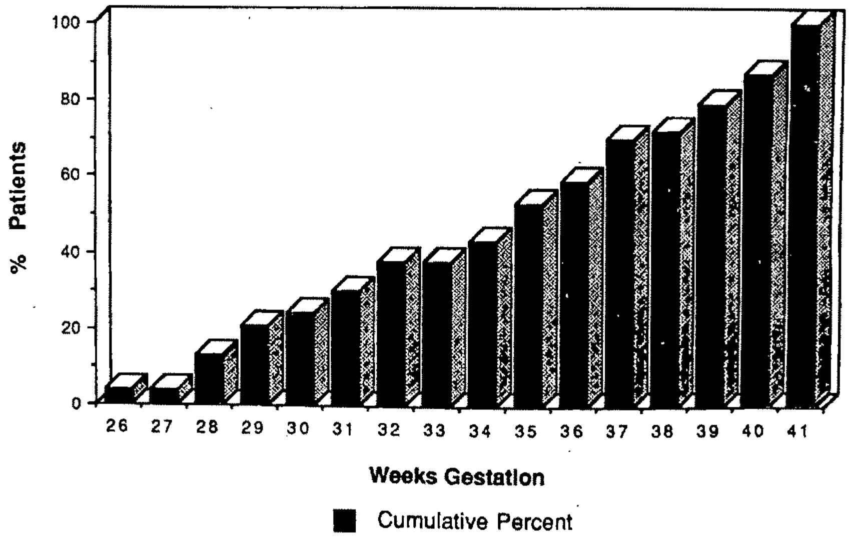

Fig. 1. Cumulative percentage of all patients with positive contraction stress test results by gestational age when first positive contraction stress test result occurred.

Table II. Neonatal deaths in patients with chronic hypertension or nonproteinuric pregnancy-induced hypertension

Patient	*GA (wk)*	*Weight (gm)*	*SGA*	*Labor and delivery*	*Apgar scores*	*Neonatal diagnosis*
1	26	420	Yes	SPROM, CS (fetal distress), renal failure	4-9	RDS
2	28	850	No	CS (maternal indications), BP 190/120	5-8	RDS
3	33	1450	Yes	Twin, PTL, CS (fetal distress)	0-2	RDS, MAS
4	33	1760	Yes	SPROM, CS (breech)	1-5	RDS, Trisomy 13
5	39	3710	No	NSVD	8-9	Anomalies

GA, Gestational age; *weight,* birth weight; *SPROM,* spontaneous premature rupture of membranes; *CS,* cesarean section; *RDS,* respiratory distress syndrome; *PTL,* premature labor; *MAS,* meconium aspiration syndrome; *NSVD,* normal spontaneous vaginal delivery; *BP,* blood pressure.

meconium aspiration syndrome. The other two were attributed to anomalies. One of the deaths secondary to respiratory distress syndrome occurred in an infant delivered at 28 weeks' gestation because of severe maternal disease (blood pressure, 190/120). However, none of the neonatal deaths occurred after intervention for abnormal antepartum fetal test results. Thus there were a total of six perinatal deaths in those patients who underwent antepartum fetal tests, giving an uncorrected perinatal mortality rate of 6.5/1000.

Of the 917 patients tested, 53 (5.7%) had at least one positive contraction stress test result. Of the 53 patients with a positive test result, 23 (43%) occurred before 35 weeks' gestation. The cumulative percentage of positive contraction stress test results that occurred at or before a given gestational age is shown in Fig. 1. The first positive test results occurred at 26 weeks' gestation.

Similar results were found when examining the data of those patients delivered of infants because of an abnormal test result. A total of 119 (13.0%) patients were delivered of infants because of an abnormal test result. Twenty-two (19%) of these patients were delivered of infants before 35 weeks' gestation. Of those who required intervention, the cumulative percentage of interventions that occurred at or before a given gestational age is shown in Fig. 2. The earliest a patient was delivered of an infant because of an abnormal antepartum test result was 26 weeks' gestation.

Comparisons were made in an attempt to identify those patients whose contraction stress test result were positive before 35 weeks' gestation or who had intervention before 35 weeks' gestation because of an abnormal test result. Patients with antepartum fetal test evidence of early compromise could not be identified by maternal age or diastolic or systolic blood pressure levels at the time of testing.

There were 114 patients who were delivered of SGA infants. This included 55 patients with chronic hypertension and 59 patients with nonproteinuric pregnancy-induced hypertension. Fourteen of the 53 patients

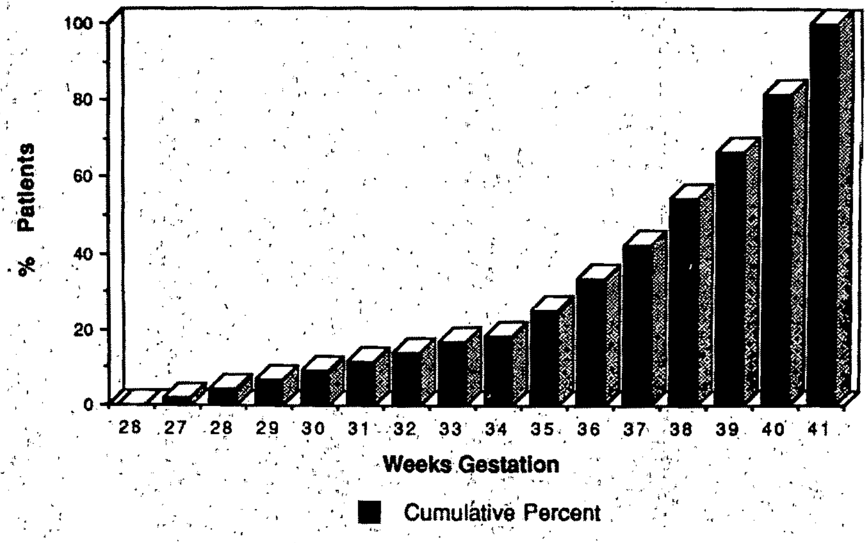

Fig. 2. Cumulative percentage of all patients who required delivery because of abnormal antepartum test results by gestational age when delivery occurred.

(26%) with a positive contraction stress test result and 29 of the 119 patients (24%) delivered of infants because of abnormal test results had SGA infants. The mean birth weight percentiles of the various groups are shown in Tables III A, III B, and III C. There was no statistical difference in birth weight percentile between those study patients tested for hypertension and those tested for all other reasons during the study interval. However, among hypertensive patients, those patients who had abnormal test results early in pregnancy (<35 weeks' gestation) had lower mean birth weight percentiles than those who first had abnormal test results at or after 35 weeks' gestation (Table III B and III C).

Of the 22 patients who required intervention for abnormal antepartum test results before 35 weeks' gestation, 15 (68%) had IUGR and eight (36%) had superimposed preeclampsia. Only five (22%) with intervention for abnormal antepartum test results before 35 weeks' gestation had neither IUGR nor superimposed preeclampsia.

Patients with positive contraction stress test results and intervention because of abnormal test results were further reviewed with respect to coexistent medical diagnoses. Of the 53 hypertensive patients with a positive contraction stress test result, 12 had a diagnosis of diabetes mellitus. This included four patients with class A diabetes. There was no statistically significant difference in the incidence of a positive contraction stress test result in diabetic patients when compared with nondiabetic patients (8.9% vs 5.3%). However, when a diabetic patient had a positive contraction stress test result it occurred at a gestational age of <35 weeks with greater frequency than in those patients without diabetes (Fig. 3). A similar trend was seen when reviewing those patients with intervention because of abnormal antepartum test results (Fig. 3). However, this difference did not reach statistical significance. Similar results were seen when reviewing patients with a concomitant diagnosis of systemic lupus erythematosus (Fig. 4). The earliest gestational age at which intervention occurred as a result of abnormal test results in patients with diabetes or systemic lupus erythematosus was 28 and 26 weeks' gestation, respectively.

Those patients with evidence of early compromise (a positive contraction stress test result or intervention because of an abnormal antepartum fetal test result at <35 weeks' gestation) were then evaluated for evidence of IUGR or a concomitant diagnosis of diabetes mellitus or systemic lupus erythematosus. Of the 23 patients who had positive contraction stress test results before 35 weeks' gestation, only three patients did not have one of these three diagnoses. Similarly, only three of 22 patients with early intervention (<35 weeks' gestation) did not have one of these three risk factors. One of three remaining patients had superimposed preeclampsia. Patients who required intervention because of an abnormal antepartum test result without IUGR, systemic lupus erythematosus, or diabetes were then studied. The cumulative percentage of patients without IUGR, systemic lupus erythematosus, or diabetes who required intervention at or before a given gestational age is shown in Fig. 5.

Comment

It has been long recognized that patients with hypertensive disease are at risk of intrauterine fetal death. For this reason, antepartum fetal heart rate testing or biophysical profile testing has generally been recom-

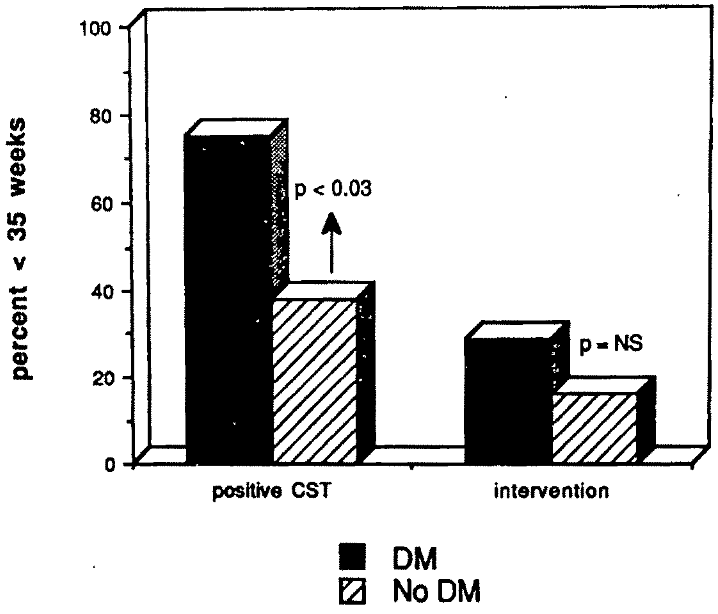

Fig. 3. Percentage of hypertensive patients with diabetes compared with those without diabetes who had either positive test results before 35 weeks' gestation or required intervention before 35 weeks' gestation.

mended in these patients.[8,9] Indeed, in this study there was only one fetal death among all 917 hypertensive patients in the group with antepartum fetal tests, whereas there were nine among hypertensive patients who did not have fetal testing during the same time interval. Although there have been considerable data to support the benefits of these testing methods, there are little data to guide the clinician as to when to start testing.

Ideally testing should be initiated before evidence of fetal compromise. The onset of compromise is variable and dependent on the clinical situation. Most reports have recommended that testing be started between 32 and 36 weeks' gestation.[1-3] However, there are patients who show evidence of compromise before this gestational age. In an attempt to identify those patients who demonstrate compromise earlier in pregnancy, we examined pregnancy outcomes and antepartum test results in patients with a primary diagnosis of chronic hypertension or nonproteinuric pregnancy-induced hypertension. We defined compromise as stillbirth, positive contraction stress test result, or intervention because of any abnormal antepartum test result (contraction stress test, nonstress test, biophysical profile). A significant percentage of patients had at least one positive contraction stress test result (5.3%) or intervention because of an abnormal test result (13.0%). Forty-three percent of those with a positive contraction stress test result had their first positive test result before 35 weeks' gestation and 18% of those who required intervention because of an abnormal test result did so

Table III A. Birth weight by percentile

	Weeks' gestation	*Mean weight percentile*	*p Value*
All patients	>25	51.3 ± 25.8	
Chronic hypertension	>25	53.0 ± 25.7	NS
Nonproteinuric PIH	>25	49.8 ± 26.8	NS

PIH, Pregnancy-induced hypertension.

Table III B. Birth weight by percentile for patients with positive contraction stress test results

	Weeks' gestation	*Mean weight percentile*	*p Value*
Chronic hypertension	<35	14.3 ± 6.4	<0.001
Chronic hypertension	≥35	50.3 ± 27.8	
Nonproteinuric PIH	<35	17.9 ± 4.8	<0.001
Nonproteinuric PIH	≥35	47.5 ± 23.7	

PIH, Pregnancy-induced hypertension.

Table III C. Birth weight by percentile for patients with intervention because of abnormal test results

	Weeks' gestation	*Mean weight percentile*	*p Value*
Chronic hypertension	<35	14.6 ± 6.9	<0.001
Chronic hypertension	≥35	51.3 ± 25.0	
Nonproteinuric PIH	<35	9.6 ± 9.3	<0.001
Nonproteinuric PIH	≥35	60.3 ± 22.4	

PIH, Pregnancy-induced hypertension.

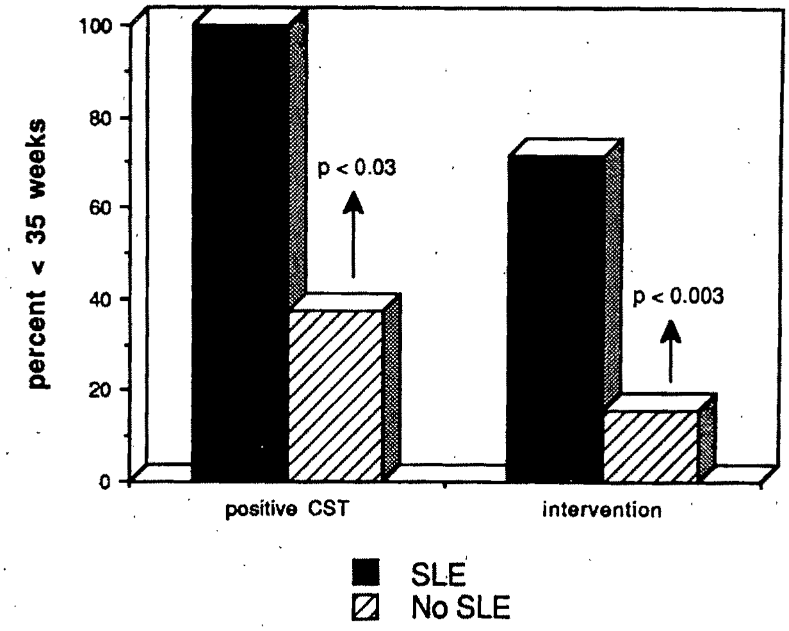

Fig. 4. Percentage of hypertensive patients with systemic lupus erythematosus compared with those without systemic lupus erythematosus who had either positive test results before 35 weeks' gestation or required intervention before 35 weeks' gestation.

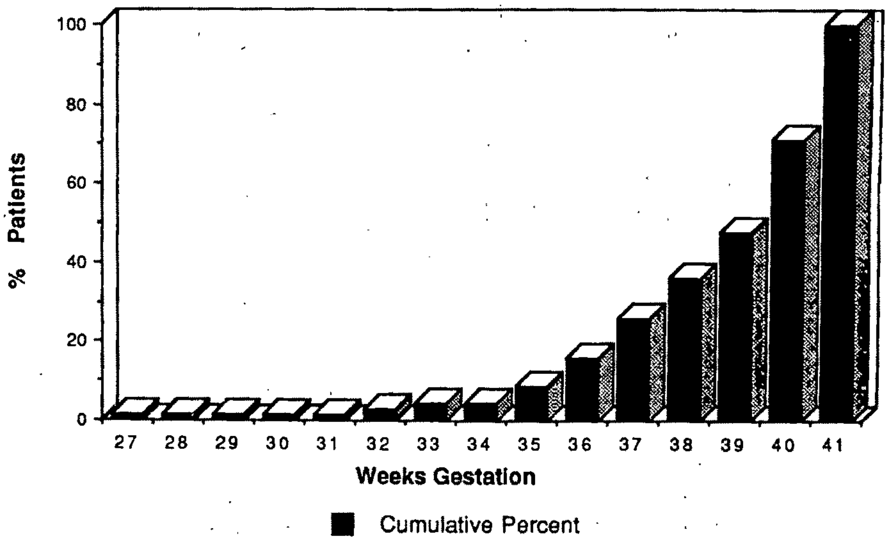

Fig. 5. Cumulative percentage of all hypertensive patients without IUGR, systemic lupus erythematosus, or diabetes who required delivery because of abnormal antepartum test results by gestational age when delivery occurred.

before 35 weeks' gestation. Therefore we cannot support the recommendation that testing be started at 35 weeks' gestation in all patients with a diagnosis of chronic hypertension or nonproteinuric pregnancy-induced hypertension.

Recognizing this problem, recent reviews have suggested that testing be started earlier in patients with risk factors such as poorly controlled blood pressure.[1-3] This recommendation cannot be substantiated by our data. There was no correlation between those who demonstrated compromise before 35 weeks' gestation and the systolic or diastolic blood pressure level at the time of testing. Furthermore maternal age did not correlate with the onset of fetal compromise.

Another suggestion has included earlier testing in those patients with evidence of IUG or superimposed preeclampsia. Although a substantial number of patients with early compromise were delivered of a growth-retarded neonate, approximately one third of patients with evidence of early compromise were delivered of a normally grown infant. Similarly, superimposed preeclampsia was not evident in all patients with early compromise.

In patients with chronic hypertension or nonproteinuric pregnancy-induced hypertension, diabetes or systemic lupus erythematosus appear to place the patient at additional risk of early compromise in pregnancy. These patients demonstrated compromise before 35 weeks' gestation with greater frequency when compared with those without these diagnoses. When coupled with evidence of IUGR and superimposed preeclampsia these factors identified more than 90% (20/22) of patients who required early intervention because of abnormal test results.

Patients with evidence of IUGR and patients with systemic lupus erythematosus required delivery because of abnormal antepartum test results as early as 26 weeks' gestation. Therefore in patients with hypertension, an obstetric ultrasonographic examination for interval growth at approximately 26 weeks' gestation would be helpful to identify the growth-retarded fetus. Testing in patients with evidence of growth retardation as well as in patients with systemic lupus erythematosus needs to be started when fetal viability is sufficient to consider intervention (as early as 25 to 26 weeks' gestation in our hospital). Similarly the earliest intervention in a hypertensive patient with diabetes was 28 weeks' gestation. Two patients with diabetes and hypertension had stillbirths in the late phase of the second trimester. Therefore testing should be started at viability in this patient population as well.

If patients with IUGR, systemic lupus erythematosus, and diabetes are excluded, the cumulative percentage of patients who required intervention because of abnormal test results at or before a given gestational age is shown in Fig. 5. One can see that there are only a few patients who demonstrate early compromise (<35 weeks' gestation) without the noted risk factors. Furthermore, if one reviews those patients with an intrauterine fetal death who had no testing and excludes those with diabetes and IUGR, then all the remaining patients experienced the loss after 33 weeks' gestation. Therefore we recommend to start testing at 33 weeks' gestation in those patients with chronic hypertension or nonproteinuric pregnancy-induced hypertension but without systemic lupus erythematosus, IUGR, diabetes, or superimposed preeclampsia. Whereas this recommendation will inevitably miss a rare patient with earlier compromise, early testing will be avoided in the majority of patients with chronic hypertension or nonproteinuric pregnancy-induced hypertension.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. MALCOLM MARGOLIN, Los Angeles, California. It is well known that patients with hypertensive disease are at risk of intrauterine fetal death.[1] Historically two thirds of fetal deaths occur before the onset of labor.[2] To reduce this risk, fetal surveillance must begin before this time. The goal of such surveillance is the detection of potential fetal compromise so that measures may be taken to prevent adverse fetal outcome.[3] The literature is replete with reports documenting the benefit of antepartum fetal testing in reducing the stillborn rate in the high-risk pregnancy.[1, 2] However, there is no universally agreed on time at which such surveillance should begin.

Drs. Pircon, Freeman et al. have reviewed a group of pregnant patients with chronic hypertension and nonproteinuric pregnancy-induced hypertension who presented over a 12-year period. There were 10 patients with stillborn fetuses included in the study. Nine of these patients did not have antepartum fetal testing because they first presented with fetal death. There were 917 patients who had antepartum fetal testing. Of these patients there was only one stillborn fetus and this occurred in a patient with superimposed preeclampsia. In the entire study group there was only one patient who had a stillborn fetus and who had no additional risk factor aside from hypertension.

A total of 119 patients were delivered of infants because of abnormal antepartum test results. Of these 119 patients, 22 were delivered of infants before 35 weeks' gestation. Most of these patients had a concomitant diagnosis of systemic lupus erythematosus, IUGR, diabetes mellitus, or preeclampsia.

The authors reviewed patients in various subgroups in an attempt to identify the time when antepartum testing in these high-risk patients should be initiated. They found that if tests were initiated at 35 weeks' gestation they would miss 18% of patients requiring intervention because of an abnormal test result. However, if all patients had testing initiated at 26 weeks' gestation (viability) a large number of unnecessary tests at significant financial and manpower costs would be required. For example, the costs of these tests at my hospital are as follows: nonstress test, $50; contraction stress test, $80; and biophysical profile, $200. On further analysis of their patient groups they found that if they began testing at 33 weeks' gestation in patients with hypertension or nonproteinuric pregnancy-induced hypertension only (i.e., patients without other concomitant diagnosis), they would identify the vast majority of patients who would require early intervention. They then defined additional subgroups of high-risk patients who require earlier testing, these being hypertensive patients with a concomitant diagnosis of systemic lupus erythematosus, IUGR, diabetes mellitus, or superimposed preeclampsia. Their recommendation is for testing to begin at fetal viability (i.e., 26 weeks' gestation) in patients with these diagnoses but for all other testing to begin at 33 weeks' gestation.

It is reemphasized by the findings in this article that it is essential for hypertensive pregnant patients to have glucose screening, an ultrasonographic examination at 26 weeks' gestation to rule out IUGR, and an evaluation to rule out the presence of collagen vascular disorders.

I want to congratulate the authors on their continued fine work in the area of attempting to define the parameters of antepartum fetal surveillance. In this age of cost containment it is essential to limit unnecessary testing while remaining cognizant of patients at reasonable risk of increased perinatal mortality. If all patients in this study had testing started at 26 rather than 33 weeks' gestation there would have been an average of seven more tests required per patient or a total of 6419 additional tests required at the very least. At an average cost of $80 per test there would be a differential of $513,520 which is surely significant. However, because many of the patients in this study had concomitant diagnoses that required earlier testing, the savings might not have been so significant. I would like to ask the authors how many of the 917 hypertensive patients studied had concomitant diagnoses that by their recommendations would have required earlier testing? If we review those patients with stillborn fetuses we find that only one patient did not have an additional risk factor.

Because antepartum death is not the only end point in which we are interested, I would also like to know what the perinatal mortality was in this group of patients. Of particular interest would be the outcome of the 119 patients who underwent early delivery because of abnormal antepartum fetal test results and how the outcome of those delivered of infants before 35 weeks' gestation compared with those delivered of infants after that time.

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DR. HOWARD A. BLANCHETTE, Berkeley, California. Dr. Freeman, you are the father of the contraction stress test. What is the role of the nonstress test in the evaluation of these patients?

DR. E. PAUL KIRK, Portland, Oregon. In the list of stillbirths, it seemed that there was a high average maternal age with the exception of one 16-year-old patient. The latter is a little bit surprising. Might maternal age be a variable in the group of patients without other complications that might lead you to adjust the recommendations for starting antepartum testing?

DR. FREEMAN (Closing). Dr. Margolin, the numbers of patients with various complications who would have required testing before 32 weeks' gestation were: 145 with diabetes, 114 with IUGR, and 11 with lupus erythematosus. The time of onset of superimposed preeclampsia was quite variable and usually occurred just about the time of intervention, so I do not know what costs a policy of earlier testing would have added.

The overall uncorrected perinatal mortality was 6.5/1000: one stillbirth and five neonatal deaths. Analysis of the neonatal deaths revealed that none were a result of intervention because of an abnormal antepartum fetal test result. Two were caused by anomalies, two followed premature deliveries for other indications, and one resulted from premature delivery for maternal indications.

Dr. Blanchette, I am always happy to talk about contraction stress tests and nonstress tests. We do not use a contraction stress test any more for primary surveillance. We use a modified biophysical profile, which consists of nonstress test and an amniotic fluid index. We do that on a twice-weekly basis. We have used the modified biophysical profile for the past 2 years for primary fetal surveillance. We had no fetal deaths with that approach. I think the reason that the modified biophysical profile is better than the nonstress test alone is because the nonstress test alone is only an acute monitor. The modified biophysical profile with the use of the amniotic fluid volume contains a chronic marker as well. The one place where we do not use that approach is in diabetic patients; we still use the contraction stress test because the amniotic fluid volume of diabetic patients is not an accurate marker. Diabetic fetuses can die with normal amniotic fluid volume.

Dr. Kirk, my coauthor Dr. Pircon looked at the maternal age along with a number of other factors and was unable to find a correlation between maternal age, positive contraction stress test results, or the need for intervention. Maternal age correlated with stillbirths, but primarily in nontested patients. Maybe that says that there is a group of older patients who do not get tested for some reason.

The roles of ultrasonography and amniocentesis in evaluation of elevated maternal serum α-fetoprotein

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A total of 681 pregnant women were referred for evaluation of elevated maternal serum α-fetoprotein levels. Ultrasonographic examination yielded an explanation for the elevation of maternal serum α-fetoprotein in 42% of patients. Diagnoses made by ultrasonography included incorrect fetal dating, multiple gestation, fetal death, open neural tube defect, abdominal wall defect, placental abnormalities, cystic hygroma, renal anomalies, and oligohydramnios. Optimal prenatal diagnosis of fetal anomalies also requires the use of amniocentesis in many patients. Amniocentesis may be obviated if fetal dating is incorrect, if an unsuspected multiple gestation is discovered, or if there is a clear anomaly and the parents do not desire genetic counseling based on karyotype information. If the fetus appears normal, the ultrasonographic results are equivocal, or the parents desire more detailed genetic counseling when an anomaly is found by ultrasonography, then amniocentesis should be performed. Thirteen abnormalities were diagnosed by amniocentesis alone in this group. (AM J OBSTET GYNECOL 1991;164:1571-6.)

Key words: Maternal serum α-fetoprotein, amniocentesis, fetal ultrasonography

The California Maternal Serum α-Fetoprotein Screening Program offers prenatal testing aimed at detection of open neural tube defects and other congenital anomalies. The program began in 1986 in response to legislation that required all pregnant women be offered maternal serum α-fetoprotein (AFP) testing between 15 to 20 weeks' gestation. Women with abnormal maternal serum AFP levels are referred to state-approved prenatal diagnostic centers for counseling, ultrasonography, and possible amniocentesis by persons with known expertise in these areas.

Ultrasonography plays a vital role in the evaluation of patients with elevated maternal serum AFP levels. Such elevations can be a result of open neural tube defects, but they can also be caused by incorrect fetal age, multiple gestation, fetal death, abdominal wall defects, cystic hygroma, renal anomalies, intestinal atresias, and other anomalies.[1, 2] Most of these conditions can be identified ultrasonographically.

This article reviews the roles of ultrasonography and amniocentesis in the evaluation of patients with elevated maternal serum AFP levels at our center.

Material and methods

During the period from April 1986 through December 1989, 681 pregnant patients were referred for early midtrimester ultrasonography and possible amniocentesis because of elevated maternal serum AFP levels. All maternal serum AFP testing was performed during 15 through 20 weeks' gestation, as determined by the patient's last menstrual period. An elevated maternal serum AFP level was defined by the State of California as one that exceeded 2.5 multiples of the median (MOM) for menstrual age for singleton pregnancies. For known twin gestations an elevated maternal serum AFP level was defined as one that exceeded 3.5 MOM before February 12, 1987, and one that exceeded 4.5 MOM after that date. The median maternal serum AFP level is determined by certified laboratories of the State of California.

Accurate fetal age was determined by biparietal diameter or femur length in all cases. The number of gestations was also determined ultrasonographically. If the maternal serum AFP level was within normal range after correction for ultrasonographically determined fetal age and number of gestations or if the fetal age was too advanced for inclusion in the program, a full obstetric ultrasonographic examination was performed. If the maternal serum AFP level was truly elevated after correction, a detailed ultrasonographic examination and/or amniocentesis was performed. These sonograms were performed by sonologists experienced in fetal ultrasonography. Fetal anatomy was examined in detail. Measurements for fetal evaluation and fetal dating were obtained; these included biparietal diameter,

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6/6/27948

Table I. Ultrasonograhic diagnoses in patients referred for elevated maternal serum AFP*

Diagnosis	*No. of patients (n = 681)*	*% of Total patients*
Incorrect fetal age	120	18
Multiple gestation	64	9
Fetal death	33	5
Open neural tube defect	31	5
Abdominal wall defect	20	3
Oligohydramnios	22	3
Placental abnormalities	21	3
Cystic hygroma	4	0.5
Renal anomalies	2	0.3
Cystic adenomatoid malformation	2	0.3
Cerebral ventriculomegaly	1	0.1
Agenesis of the corpus callosum	1	0.1
Normal	373	55

*Patients may have more than one diagnosis.

head circumference, abdominal circumference, and femur length. Fetal position, amniotic fluid volume, and placental position and structure were also noted.

Full fetal ultrasonographic examinations were performed with a variety of real-time scanners during the first 6 months of the screening program. After this initial period, detailed examinations were performed only on an Acuson 128 scanner (Mountain View, Calif.) with transducer frequencies from 3.0 to 5.0 MHz.

In cases in which the fetal sonogram showed an anomaly to account for the elevated maternal serum AFP level, amniocentesis was performed if the anomaly was associated with chromosomal aberrations or if the patient desired such information for use in genetic counseling for consideration of future pregnancies. In consenting patients, amniocentesis was always attempted if the fetus appeared normal ultrasonographically or if the ultrasonographic results were equivocal.

The amniotic fluid AFP level was determined by our laboratory. If this level exceeded 2.0 MOM for menstrual age, samples were sent to an independent laboratory (University of California, Los Angeles Medical Center) for repeat amniotic fluid AFP analysis and for determination of the presence of acetylcholinesterase. This protocol was mandated by the State of California. Amniotic fluid AFP levels more than 2.0 MOM were considered elevated. Acetylcholinesterase was reported as not present, weakly positive, or strongly positive.

All patients were counseled with regard to the significance of ultrasonographic findings and amniocentesis results. Therapeutic options were offered. If the pregnancy was terminated, fetal pathologic findings were correlated with the results of ultrasonography and amniocentesis. If the pregnancy was continued, information on the outcome was obtained from the referring physician and was correlated with prenatal tests.

Results

Ultrasonographic results. Six hundred and eighty-one patients with elevated maternal serum AFP levels had ultrasonography with more detailed sonograms performed in 520 patients. A total of 137 ultrasonographic abnormalities were detected (Table I).

There were seven fetal anomalies diagnosed in 64 twin pregnancies. Open spina bifida was detected ultrasonographically in one twin of a diamniotic-dichorionic pregnancy. Two other sets of twins had one fetus affected with an abdominal wall defect, one omphalocele and one gastroschisis. One set of twins had both twins affected with gastroschisis, which has been previously reported.[3] There was one set of twins with one live twin and one dead twin and in one set of twins, one twin was affected with crossed-fused renal ectopia.

In the group of 33 patients with fetal death there were eight patients who had additional related diagnoses seen by sonogram: three cases of oligohydramnios, one cerebral ventriculomegaly, and one encephalocele, one live co-twin, one abruptio placentae, and one cystic hygroma.

Twenty-nine diagnoses of open neural tube defects were made by ultrasonography. They were 17 anencephalic fetuses, four with encephalocele, and eight with open spina bifida. In two additional patients, the sonograms were equivocal for the diagnosis of open spina bifida.

Twenty abdominal wall defects were diagnosed by ultrasonography in the referred patients. Ten were gastroschisis, eight were omphaloceles, one was an omphalocele with other anomalies, and one was a limb-body wall complex.

In the 22 cases of oligohydramnios there were three fetal deaths and one fetus had an omphalocele. In the remainder of cases the examination by ultrasonography was limited but no definite anomalies were seen.

Among those 21 patients with placental abnormalities seven had placenta previa, seven had abruptio placentae, four had venous lakes, and three had subchorionic hematomas.

Other anomalies diagnosed by ultrasonography in this series are listed in Table I.

Amniocentesis results. Amniocentesis was performed on 442 (65%) patients. It was not offered to those patients whose maternal serum AFP level fell within normal range when corrected for fetal age or multiple gestation or whose dates were too far advanced for elective options ($n = 161$). Amniocentesis failed in seven patients with oligohydramnios. The procedure was refused by the remaining 71 patients.

Among the 442 patients who underwent amniocentesis, 13 additional confirmed abnormalities were diagnosed (Table II).

Nine chromosomal anomalies occurred in this series. Four of these occurred in conjunction with other ultrasonographically diagnosed abnormalities. One XO

(Turner's syndrome) karyotype was detected in a fetal death. Two of the fetuses with encephaloceles had chromosomal aberrations: one unbalanced 21/21 translocation and one trisomy 18. One fetus with an abdominal wall defect had a trisomy 18.

Five chromosomal anomalies occurred in patients with normal sonograms. There were three significant (47, XYY; mosaic XO; 46,X + centromeric fragment) and two were minor (familial inversion, 46,X with inverted Y).

Also among patients with normal sonograms there were three cases in which the amniotic fluid AFP was significantly elevated and the acetylcholinesterase was strongly positive; all three had open spina bifida confirmed after elective abortion.

Amniocentesis results were abnormal in five other patients with normal sonograms; in all five cases the fetuses were normal. In four of these cases the amniotic fluid AFP levels were mildly elevated (less than 3 MOM) and the acetylcholinesterase results were negative; these pregnancies were continued with normal outcomes. In the fifth case the amniotic fluid AFP was markedly elevated (20 MOM) and the acetylcholinesterase level was weakly positive but the fluid had been slightly bloody. Although the patient was advised to undergo repeat amniocentesis, she elected to terminate the pregnancy and a normal fetus was seen on pathologic examination.

In the remaining 424 patients who underwent both amniocentesis and ultrasonography there was appropriate correlation between the two tests. Nineteen of the ultrasonographically detected open neural tube defects were confirmed by amniocentesis; all were strongly positive for acetylcholinesterase and had significantly elevated amniotic fluid AFP levels. Ten cases of anencephaly did not undergo amniocentesis. In two cases in which the ultrasonographic diagnosis of open spina bifida had been equivocal, amniocentesis results were normal and outcomes were normal.

Twelve patients with fetuses with abdominal wall defects underwent amniocentesis. In all, the acetylcholinesterase level was at least weakly positive and amniotic fluid AFP level was elevated.

In the case of multicystic dysplastic kidney, the amniotic fluid AFP level was elevated but the acetylcholinesterase results were negative.

Outcome. Information on the outcome of pregnancy was obtained in 452 (87%) of the patients with maternal serum AFP levels that were persistently elevated after correction for fetal age and number of gestations. Sixty-eight patients were lost to follow-up; 59 of these had normal sonograms, four had oligohydramnios, two had placental abnormalities, one had multiple congenital anomalies including an abdominal wall defect, one had a cystic adenomatoid malformation, and one had an open spina bifida with a confirmatory amniocentesis.

Thirty-one patients with open neural tube defects underwent elective abortion; twenty-eight of these cases had been diagnosed by ultrasonography and the other three had diagnostic amniocenteses but normal ultrasonography results. In the case of the twins with one affected fetus, a selective termination of the affected twin was performed in utero with subsequent delivery of one normal infant at 34 weeks' gestation. One fetus with open spina bifida diagnosed by ultrasonography and amniocentesis was lost to follow-up after the parents decided to continue the pregnancy. Each of the two patients with equivocal sonograms for open spina bifida and normal amniocentesis results was delivered of a normal infant.

Of the 20 cases of abdominal wall defects, 14 were terminated; the one fetus with concomitant oligohydramnios died in utero. Five patients continued their pregnancies including the patient with a twin pregnancy in which both fetuses had gastroschisis. Both twins are alive and well after neonatal surgery to correct the defects. One fetus with an abdominal defect and multiple other anomalies was lost to follow-up; in all other patients the ultrasonographic and amniocentesis diagnoses were confirmed.

In the group of 22 patients with oligohydramnios there were six normal singleton babies and one set of normal twins delivered. There were five spontaneous

Table II. Confirmed abnormalities diagnosed by amniocentesis alone

Abnormality	*No. of patients (n = 442)*
Chromosomal	
With additional abnormalities seen ultrasonographically	4
With normal sonograms	
Significant	3
Minor	2
Open spina bifida	3
Hamartoma	1
TOTAL	13

Table III. Outcome of pregnancy in patients with elevated maternal serum AFP and normal detailed fetal sonograms*

Outcome	*Normal amniocentesis results*	*Abnormal amniocentesis results*
Normal outcome	316	4
Abnormal baby at birth	5	4
Stillbirth	4	0
Spontaneous abortion	5	0
Elective abortion of normal fetus	0	1
Elective abortion of abnormal fetus	0	5
Neonatal death	3	0

$n = 347$.

*Excludes patients who refused amniocentesis or were lost to follow-up.

abortions, four elective abortions, one neonatal death after a premature delivery, and one baby with a club foot that was not detected prenatally. Four patients were lost to follow-up.

In the 21 patients with placental abnormalities diagnosed by ultrasonography, there was one spontaneous abortion and one premature delivery, both in patients with abruptio placentae. Two patients were lost to follow-up and the remainder had normal outcomes.

The remaining abnormalities diagnosed by ultrasonography were confirmed either at birth or by pathologic examination at termination in those cases with follow-up.

Outcomes in the group with normal fetal sonograms are listed in Table III. There were five elective abortions performed on the basis of amniocentesis results that yielded abnormal fetuses—three cases of open spina bifida, one hamartoma, and one XYY chromosomal anomaly. Infants were delivered in the four other cases of chromosomal abnormalities diagnosed by amniocentesis.

Congenital anomalies not diagnosed by ultrasonography or amniocentesis included one patient with congenital hypothyroidism, two with hypospadias, and two with cleft lip.

In the group with normal ultrasonography and normal amniocentesis results, there were five spontaneous abortions including one that occurred 3 days after amniocentesis. This constitutes a fetal death rate of 0.2% from amniocentesis. There were four stillborn infants and three neonatal deaths.

There was one elective abortion of a normal fetus (described in this article) after amniocentesis revealed an elevated amniotic fluid AFP level (20 MOM) and a weakly positive acetylcholinesterase level.

Comment

The results of this series support a complementary role for ultrasonography and amniocentesis in the evaluation of patients referred with elevated maternal serum AFP levels. Other series have also supported the conclusion.[4]

Although the primary goal of the California Maternal Serum α-Fetoprotein Screening Program is the detection of open neural tube defects, in this series only 5% of patients referred had such abnormalities. Fourteen percent of patients referred had other abnormalities diagnosed by ultrasonography or amniocentesis.

Ultrasonography was 90% sensitive in the diagnosis of open neural tube defects in this series. Published series have shown similar sensitivities.[5] There were three false-negative diagnoses by ultrasonography. Use of amniocentesis as a complementary procedure improved the sensitivity of detection of open neural tube defects to 100%. The three false-negative cases of open spina bifida occurred during the first 6 months of our screening program and, as reported previously, changes in scanning protocols[6] as well as more recently described intracranial ultrasonographic signs associated with spina bifida[7, 8] appear to have increased our accuracy in the ultrasonographic diagnosis of this anomaly.

Ultrasonography may be technically difficult because of such factors as maternal body habitus, fetal lie, fetal motion, and oligohydramnios. In such cases the examination may lead to equivocal results. As in the two equivocal cases reported here, amniocentesis may rule out the diagnosis of open spina bifida.

Conversely, it must be remembered that amniocentesis results may also be equivocal. In four patients in this series amniotic fluid AFP results were elevated but less than 3 MOM and acetylcholinesterase results were negative. All four patients had extensive fetal ultrasonographic results that showed no abnormality and all four patients were delivered of normal babies.

A more difficult situation arises in the case of a normal sonogram when the amniotic fluid AFP level is significantly elevated but the acetylcholinesterase determination does not unequivocally support the diagnosis of a neural tube defect. There were two such cases in this series and both pregnancies were terminated; one fetus had a hamartoma at the base of the spine whereas the other fetus was normal. Perhaps repeat detailed ultrasonographic examination after amniocentesis results are known and at a more advanced fetal age would help to differentiate such cases in the future.

Chromosomal aberrations without other fetal anomalies have been reported in patients seen for evaluation of elevated maternal serum AFP levels.[9] Chromosomal aberration can also be associated with most of the anomalies diagnosed by ultrasonography in this series.[10, 11] We had nine cases of chromosomal abnormalities diagnosed in this series. Four were associated with ultrasonographically visible abnormalities and five were not. Such information is useful not only in evaluation of the current fetus but also can be helpful in genetic counseling if future pregnancies are desired.

In this series one spontaneous abortion occurred 3 days after amniocentesis and may have been attributable to the procedure. This constitutes a fetal death rate of 0.2% from amniocentesis. Although there is a risk of fetal death because of amniocentesis, this appears to be low enough to justify use of the procedure to increase prenatal diagnostic accuracy in 3% of cases.

The remainder of the spontaneous abortions in patients with normal ultrasonographic results occurred more than 1 week after amniocentesis. This group and those with stillbirths and neonatal deaths are considered to be reflective of the increased risk of abnormal outcome in patients with elevated maternal serum AFP levels and normal ultrasonographic and amniocentesis results.[5, 12, 13]

A maternal serum AFP screening program can be highly effective in the prenatal diagnosis of fetal anomalies. Such a screening program requires close cooperation between the sonologist, the obstetrician, and the geneticist for optimal prenatal diagnosis and care. Fetal ultrasonography can frequently determine the cause of an elevated maternal serum AFP level and should be the first examination used in the evaluation of patients with maternal serum AFP elevations. If a clear structural anomaly is visualized on fetal ultrasonography and the parents do not wish genetic counseling based on chromosome analysis for consideration of future pregnancies, then amniocentesis can be obviated. Patients with truly elevated maternal serum AFP levels who have normal fetal sonograms or who have abnormal sonograms and desire counseling should undergo amniocentesis.

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Discussion

DR. LOUIS BARTOLUCCI, San Francisco, California. Dr. Hanson reports again on the results of the California Maternal Serum α-Fetoprotein Screening Program at the University of California, Davis Medical Center. The study now spans a period of 2½ years and concerns 681 patients referred to that institution for levels of maternal serum AFP over 2.5 MOM in singletons and 4.5 MOM in twins.

I am happy to see that his protocol now includes basic and detailed ultrasonographic studies before invasive procedures are advised. This is a welcome change from the earlier protocol that prescribed level II ultrasonography only if amniotic fluid AFP levels were found abnormal.[1]

The main concern I have about this important study and its implications for our patients has to do with the unequivocal statement made by Dr. Hanson et al. that patients with truly elevated maternal serum AFP levels who have normal fetal sonograms should undergo amniocentesis.

What is proposed and in effect mandated by the State of California is to advise patients to undergo an invasive procedure to increase the sensitivity of the maternal serum AFP screening test. The same position is generally taken by similar programs around the country at this time.[2, 3]

I wonder whether we should review this position in light of the advances made by ultrasonography in instrumentation and operator skill.

The attending obstetricians are faced with patients who have been alarmed by a screening test that unexpectedly assigned their pregnancy to a high-risk category. If they are to be fair in their counseling they must know well the risks and benefits of the course of action advised.

The predictive value of an abnormal screening test, such as maternal serum AFP, in an unselected population is strongly dependent on the prevalence of the disease the test is devised to uncover.[3] The prevalence of open neural tube defects and some other conditions associated with elevated values of maternal serum AFP levels is low in this country,[4] consequently the predictive value of an abnormal maternal serum AFP level will be low even if the sensitivity and specificity of the test were high. Conversely the predictive value of a normal maternal serum AFP level is expected to be high at such low prevalence of the disease.

Amniocentesis carries a small but definite risk to the fetus[6] and, like all invasive procedures, a small risk to the mother.[7]

Ultrasonography carries only one risk in so far as we know to date—the risk of missing an anomaly.

Dr. Hanson reports no maternal complications from the 442 amniocenteses in his study and one fetal death for a 0.2% fetal complication rate. One could, however, regard the death of the normal fetus because of false-positive amniotic fluid AFP results as an amniocentesis-related death. The same objection could be raised to a lesser degree about the aborted fetus with hamartoma, a benign, generally self-correcting condition.

An attempt should be made to quantify risks and benefits for the patient and then allow her to make the final decision.

Reports from perinatology centers around the country are beginning to appear in which the advisability of amniocentesis is questioned in the presence of a normal detailed sonogram.[8]

An exemplification of this trend can be found in a recent article by Nadel et al.[9] from the Departments of Obstetrics and Gynecology and Radiology of Harvard Medical School that addresses precisely the issue of need for amniocentesis in cases of elevated maternal serum AFP levels after a negative targeted ultrasonographic study. The article concludes that because the risk of abortion as a result of amniocentesis is higher than that of missing anomalies commonly associated with high levels of maternal serum AFP, some patients may elect to forgo amniocentesis.

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DR. DURLIN E. HICKOK, Seattle, Washington. Dr. Hanson, I want to ask about the patients referred for evaluation of elevated maternal serum AFP levels who appear to have placental abnormalities. There have been several articles[1, 2] suggesting that up to 60% of such patients have evidence of subchorionic hemorrhage, placental thickening, or placental sonolucencies. Can you provide more detail about the percentage of those cases in your series that were offered amniocentesis, had amniocentesis, and had a chromosome abnormality? I understand that many of the cases of elevated maternal serum AFP levels, the so-called false-positives that are associated with placental abnormalities, may be from fetomaternal hemorrhage. Is there any way that we can be more specific in this diagnosis? Is this a broad category for which we might eliminate amniocentesis altogether?

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DR. RUSSELL K. LAROS, San Francisco, California. If my arithmetic is right, the perinatal mortality in the patients with high maternal serum AFP levels and normal ultrasonography results was about 24 per 1000. What do you recommend to the practicing obstetrician for antenatal surveillance in this group?

DR. HANSON (Closing). Dr. Bartolucci, I share your concern, especially in view of a recent article by Benacerraf.[1] I do think that we need to reexamine the necessity for amniocentesis in cases of increased maternal serum AFP levels and normal level II ultrasonographic examinations. In California we are tied to the guidelines of the State of California Genetic Disease Branch.

Dr. Laros, I think you already know the answer to your question. We have a letter that we send to referring practitioners that disturbs many of them. We recommend that patients with elevated maternal serum AFP levels, normal amniocentesis, and normal level II scans be followed up as one would any high-risk obstetric patient. The details are not specified for medicolegal purposes. This recommendation is made on the basis of an article written by Crandall's group[2] that shows increased risk of adverse pregnancy outcomes in such patients. There is a correlation with the level of AFP. The higher it is, the greater the risk.

Dr. Hickok, I do not have an answer to your question. We see many placental abnormalities, particularly placental lakes. It is a relief when the amniocentesis reveals discolored fluid. Frequently we can confirm that there has been bleeding and more than likely this is the cause of the elevated maternal serum AFP level. Of course, these patients probably should be considered high risk because they have had bleeding at some time in the past. I still do not have the confidence, though, to say that everything is okay without having the chromosomes. The number of chromosomal abnormalities in these patients is very low. Certainly we have picked up some that have had chromosomal abnormalities and have had discolored fluid and have gone on to lose the pregnancy. However, I do not know of any way with ultrasonography alone that we can say ahead of time and with total confidence that there is a placental abnormality that is the only cause of the abnormal AFP level and that we do not need to do any further tests.

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Preeclampsia as the great impostor

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In contrast with a generation ago when preeclampsia was misdiagnosed as medical or surgical disease unrelated to pregnancy, today's diagnostic errors are those that consider surgical and medical problems as either pregnancy-induced hypertension or as the hemolysis, elevated liver enzymes, and low platelet count syndrome. Eleven case histories are presented of significant medical or surgical problems that were initially diagnosed as hemolysis, elevated liver enzymes, and low platelet count syndrome or pregnancy-induced hypertension. The incorrect diagnosis of medical-surgical cases during pregnancy often meant that appropriate therapy was delayed and that the rate of iatrogenic prematurity was increased. (Am J Obstet Gynecol 1991;164:1577-81.)

Key words: Misdiagnosis, hemolysis, elevated liver enzymes, and low platelet count syndrome, pregnancy-induced hypertension

In 1975 the concept of preeclampsia as a great imitator was presented to the Pacific Coast Obstetrical and Gynecological Society. Preeclampsia (gestosis) was misdiagnosed as viral hepatitis, cholangitis, lupus erythematosus, immunogenic throbocytopenic purpura, detached retina, and gastric ulcer. Fifteen years later, as judged by cases seen in our hospitals and in review of records for possible litigation, the types of diagnostic errors have come full circle. Physicians of all specialties are being deceived into making erroneous diagnoses of pregnancy-induced hypertension or the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) when the patient actually has a disease process unrelated to pregnancy. Under these circumstances, delivery does not necessarily improve the patient's illness and worse yet, appropriate therapy is often delayed.

Summaries of some of the cases that were misdiagnosed as toxemia or HELLP syndrome are described in this article (Table I). The patients were seen over 14 years at the University of California Hospital at Davis, University of Nebraska Hospital in Omaha, University of Colorado Health Science Center in Denver, and Denver General Hospital.

Case histories

Cardiomyopathy (two cases). A 34-year-old gravida 6, para 4 woman at 33 weeks' gestation was referred because of recurrent toxemia. On admission her blood pressure was mildly elevated and she had severe orthopenia. A chest roentgenogram showed an enlarged heart and pulmonary edema. The pulmonary wedge pressure was elevated and did not respond to furosemide. Because the patient was considered to have pregnancy-induced hypertension, labor was induced with delivery of a 1850 gm infant who had a stormy neonatal course. Post partum the patient had no evidence of pregnancy-induced hypertension. The patient continued to have congestive failure and eventually a cardiac biopsy was done. This showed cardiomyopathy, probably of viral cause. She never recovered and died 7 months post partum while awaiting a cardiac transplant. The other patient was first seen in a similar fashion and recovered from cardiomyopathy.

Dissecting aortic aneurysm (two cases). A 34-year-old gravida 5, para 4 woman was referred for severe HELLP syndrome because her liver enzymes were very high (serum glutamate oxaloacetate transaminase level >3600 U) and with a fetal death. On arrival at the hospital she was somnolent and complained only of neck and chin pain. While in the examining room she became hypotensive and totally unresponsive. She was taken immediately to the operating room for possible liver rupture. At laparotomy, necrosis of abdominal contents was found. She had a dissecting aortic aneurysm beginning at the level of the ascending arch and continuing to the level of the aortic bifurcation. She died on the operating table as did the other patient.

Cocaine abuse (three cases). A 22-year-old gravida 3, para 2 woman was admitted at 24 weeks' gestation for HELLP syndrome (serum glutamate oxaloacetate transaminase level 82 U; platelet count 105,000; blood pressure, 180/118 mm Hg). The first pregnancy was normal. Urine toxicology screening showed cocaine. After 20 hours of hospitalization, all abnormalities had begun to return to normal. She was discharged 1 week later with a diagnosis of probable chronic hypertension.

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Table I. Misdiagnosed medical-surgical cases during pregnancy

Correct diagnosis	*Initial diagnosis*	*No. of cases*	*Maternal mortality (%)*	*Perinatal mortality (%)*	*Therapy delay**
Cardiomyopathy	PIH	2	50	0	Yes
Dissecting aortic aneursym	HELLP	2	100	100	No
Cocaine abuse	HELLP	3	0	0	No
Chronic renal disease	PIH	14	0	53	Yes
Acute fatty liver	HELLP	3	100	100	Yes
Gangrenous gall bladder	HELLP	4	0	0	Yes
Glomerulonephritis	PIH	2	0	0	Yes
Immune thrombocytopenia	HELLP	4	0	0	Yes
Lupus erythematosus	HELLP	3	0	100	Yes
Pheochromocytoma	PIH	2	100	100	No
Ruptured bile duct	HELLP	1	0	0	Yes

PIH, Pregnancy-induced hypertension or preeclampsia; *HELLP,* hemolysis, elevated liver enzymes, low platelet count syndrome.
*Significant delay in appropriate medical-surgical therapy that probably increased morbidity.

At 31 weeks' gestation she was delivered of an infant prematurely, with a positive cocaine screening results liver abnormalities, and hypertension. Two other patients have been seen at 33 to 36 weeks' gestation with positive cocaine screening results and were initially considered to be examples of HELLP syndrome. With hospitalization, the abnormalities cleared without delivery.

Essential hypertension and chronic renal disease (14 cases). We reviewed the charts of 48 women younger than age 45 years who were referred for dialysis or renal transplant. It was possible to obtain obstetric care information on 14 of the patients. Of these 14 patients all were hypertensive during the pregnancies and 12 were diagnosed as having preeclampsia or pregnancy-induced hypertension. Eleven were delivered of infants before 38 weeks' gestation with only eight surviving children in the group of 15 fetuses. Whereas preeclampsia is frequently superimposed on existing vascular disease, only five charts indicated that the obstetrician recognized the seriousness of the underlying renal-vascular disease through appropriate testing. The records indicated that only two patients were counseled about the serious nature of the renal disease and provided with adequate medical follow-up.

Acute fatty liver of pregnancy with hypoglycemia (three cases). A 23-year-old gravida 1 woman with 5 years' infertility was seen on referral for twins and HELLP syndrome at 26 weeks' gestation. Laboratory work showed serum glutamate oxaloacetate transaminase values four times the upper limit of normal, a platelet count of 55,000, a serum creatinine value of 1.6 mg%, and a blood sugar level of 34 mg%. She was normotensive. Despite the marked hypoglycemia, her care was temporized with attempts to elevate the blood sugar level and she was given steroids for fetal lung maturity. Liver function became more abnormal, the hypoglycemia worsened, renal and pulmonary function deteriorated, and she suddenly died on her fifth hospital day. A postmortem cesarean section was performed; neither newborn survived. At autopsy, acute fatty liver was present. Neither of the other two patients with marked hypoglycemia survived.

Gangrenous or ruptured gall bladder (four cases). A 25-year-old multigravida was seen in the first trimester because of upper abdominal pain that was diagnosed as heart burn. The pregnancy was otherwise uneventful until 26 weeks' gestation when she was admitted to a hospital with severe right upper-quadrant pain and hypertension (a 15/40 mm Hg rise in blood pressure over a low value in the second trimester). Liver enzymes were 1.5 times the upper limit of normal and the platelet count was 125,000. A diagnosis of HELLP syndrome was made even after obtaining medical consultation. An immediate cesarean section was done. On the second postoperative day she had an emergency cholecystectomy for a gangrenous gall bladder. The mother's postoperative course was prolonged. Unfortunately, the 780 gm infant had a very stormy neonatal course and by age 2 years blindness and cerebral palsy were diagnosed. The other three patients also had postpartum cholecystectomy for ruptured or gangrenous gall bladder, but their infants had no problems.

Glomerulonephritis (two cases). A 31-year-old gravida 1 woman was referred for presumed preeclampsia at 29 weeks' gestation because she was spilling 6 to 8 gm of protein a day. Except for a low serum albumin value (2.1 mg/dl) the laboratory results were normal. Her blood pressure was moderately elevated. She was treated with daily intravenous infusions of albumin. After 2 weeks of hospitalization the pregnancy was terminated because of severe hypertension and massive proteinuria (14 to 18 gm a day). The clinical course worsened post partum and after a renal biopsy the correct diagnosis of type 1 membranoproliferative glomerulonephritis was made. She had a rapid response to corticosteroids. The other patient also responded to steroids.

Immunogenic thrombocytopenia purpura (four cases). An 18-year-old gravida 1, para 0 woman was admitted in labor at term. The initial laboratory work up showed a platelet count of 52,000. Her blood pressure was mildly elevated (144/88 mm Hg) and a diagnosis of HELLP syndrome was made. She was treated with magnesium sulfate and was subsequently delivered of an infant by cesarean section because of failure to progress. Her 3150 gm infant did well except for a platelet count of 40,000 on the third day after birth. Post partum, her platelet count declined to a low of 15,000 and a diagnosis of immunogenic thrombocytopenic purpura was made. She finally responded to a splenectomy. The other three patients responded to medical therapy.

Lupus erythematosus (three cases). A 27-year-old gravida 5 woman with no living children was referred because of severe HELLP syndrome and eclampsia at 24 weeks' gestation. The lupus, diagnosed 6 years previously, was considered to be under good control with only 15 mg of prednisone a day. Immediate past history indicated a recent grand mal seizure, severe hypertension, renal failure, thrombocytopenia, and elevated liver enzyme values. Although a serum complement value was not available, we agreed with the diagnosis of severe preeclampsia that was not responding to therapy, and the pregnancy was terminated at 24 weeks' gestation. Unfortunately, the severe lupus became obvious with continued seizures not responsive to magnesium sulfate and worsening of other symptoms. With appropriate lupus therapy, she did respond after prolonged hospitalization. The other two cases of unsuspected lupus also had inappropriate neonatal deaths.

Malignant pheochromocytoma (two cases). A 31-year-old gravida 3, para 2 woman was referred for severe pregnancy-induced hypertension at 24 weeks' gestation. In the hospital there was marked variability of blood pressure with episodes of increased vasomotor activity and the blood urea nitrogen value was 31 mg/dl. The urine showed significant increases in metanephrines and vanillylmandelic acid. Magnetic resonance imaging showed multiple nonadrenal masses. Blood pressure control with phenoxybenzamine was not successful and at 26 weeks' gestation she was delivered of a 510 gm female infant who did not survive. At the time of cesarean section, wide spread metatastic masses were found throughout the peritoneal cavity. The patient died 6 months post partum despite chemotherapy and radiation therapy. The other patient also died without a living newborn.

Ruptured bile duct (one case). A 22-year-old gravida 1 woman was transported to our hospital at 35 weeks' gestation because of severe HELLP syndrome. She was admitted with severe right upper-quadrant pain, markedly elevated liver enzymes, a platelet count of 121,000, and a white count of 34,000. Blood pressure was slightly elevated. After 8 hours of attempted induction of labor, an emergency cesarean section was performed because of fetal distress. On opening the peritoneal cavity, gross bile was encountered. A ruptured bile duct was eventually found and the patient required a Roux-en-Y hepaticojejunostomy. She was on the operating table for 9 hours and had a prolonged postoperative course. The infant was severely depressed at birth but did well.

Incomplete follow-up. Among patients with the diagnosis of HELLP syndrome during pregnancy, referring physicians have reported three patients with subsequent lupus erythematosus and four patients with immunogenic thrombocytopenic purpura some 5 to 9 years after the affected pregnancy. The patients had all been considered to have recovered completely at the time of the postpartum examination.

Comment

Lupus anticoagulant antibodies have been implicated in patients with preeclampsia and thrombocytopenia but we have not found a single positive case in screening more than 100 patients with apparent preeclampsia. Thrombotic thrombocytopenic purpura, another medical problem confused with preeclampsia, was correctly diagnosed in two patients who died. No other cases of thrombotic thrombocytopenic purpura were knowingly misdiagnosed as HELLP syndrome.

HELLP syndrome is not a new disease.[1-3] Despite the wide interest and intervention therapy in patients with HELLP syndrome, there is no evidence that the outcome of these patients has improved since Weinstein developed the acronym. Whereas the incidence of eclampsia has continued to decrease, the diagnosis of severe preeclampsia has increase.[4] In contrast with the present concept that those with the abnormal liver test results and with no or mild thrombocytopenia or hypertension are emergent cases, in the past these were usually considered cases of viral hepatitis. They were treated with bed rest and they usually had a good outcome. Even when the disease process was severe, the cases were appropriately handled.[5] Today, even with relatively mild cases, the incidence of iatrogenic prematurity has increased as a consequence of considering all grades of the HELLP syndrome as severe preeclampsia.

It is much too easy to ascribe almost any disease in pregnancy to be a result of pregnancy-induced hypertension or HELLP syndrome.[3] Previously the misdiagnosed cases of preeclampsia led to inappropriate procedures, such as cholecystectomy for right upper-quadrant pain. Although an error, this inappropriate therapy itself was seldom life-threatening. But as recounted in these case histories, to incorrectly diagnose life-threatening surgical-medical illness as HELLP syndrome can endanger the patient's life or seriously prolong the illness. The correct diagnosis would not have prevented the maternal deaths described here, but as shown in Table I, it would probably have reduced maternal morbidity and iatrogenic prematurity. It was previously suggested that obstetricians should become complete physicians and manage these patients without consultation.[1] As shown by these cases of lupus and immunogenic thrombocytopenic purpura, after a thorough evaluation is done by the obstetrician in atypical cases of HELLP syndrome it can be beneficial to the patient for the obstetrician to obtain knowledgeable consultation.

It is especially important to recognize the significance of persistent hypoglycemia in patients with possible HELLP syndrome. It indicates severe liver failure[3, 6] and immediate delivery appears to be the patient's only hope inasmuch as survivors with prolonged hypoglycemia are rare.

To assume that right upper-quadrant abdominal pain in late pregnancy is a result of HELLP syndrome without an ultrasonographic examination of the gall bladder and liver to rule out gall bladder disease or a liver hematoma can be remiss, although others consider the test to have a low yield. Gall stones can be totally unrelated to the management of HELLP syndrome but not liver hematomas.[7]

Whereas it may not always be possible to rule out immunogenic thrombocytopenic purpura and systemic lupus in these cases of suspected HELLP syndrome, an effort should be made because delivery may aggravate the clinical course. In patients with persistent or recurrent hypertension, careful follow-up should be arranged to rule out underlying renal or cardiovascular disease. Such can significantly modify the patient's life expectancy.

Steroids and low-dose aspirin have been suggested for cases of apparent HELLP syndrome that are not planned for immediate delivery.[8, 9] Even when the diagnosis of HELLP syndrome is correct, these drugs may possibly improve abnormal liver test results and platelet values of patients with preeclampsia, and when the correct diagnosis is lupus or immunogenic thrombocytopenic purpura, they can be of significant benefit.

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Discussion

DR. RALPH BENSON, Portland, Oregon. Dr. Goodlin has come full circle since his 1975 finding that the symptomatology of preeclampsia-eclampsia is often misdiagnosed as a nonobstetric problem. Today he has shown that obsetricians may fail to diagnose medical or surgical problems that masquerade as pregnancy-induced disorders. Although in the later series most parturients survived, many neonates died, usually as a result of treatment based on incorrect diagnoses or early intervention.

Unusual diseases are always intriguing. How they affect pregnancy and how pregnancy affects them may not be entirely clear to the observer. Of the 40 cases reported, 20 were initially thought to be examples of the HELLP syndrome—still presumed by some to be a potentially critical variant of severe preeclampsia-eclampsia. In Dr. Goodlin's current series, the presumptive diagnosis of the HELLP syndrome i.e., hemolysis, elevated liver enzymes, and low platelet count could not be substantiated in any instance. Accepted medical or surgical diagnoses were finally made in each case, however,

Weinstein described the HELLP syndrome in 1954. Early on, obstetricians gave this diagnosis more than tentative recognition. Nevertheless, the symptom complex was ill-defined, variable, and uncommon. Hence a thorough study of an adequate number of patients was delayed. Soon, skeptics including Dr. Goodlin began to question HELLP syndrome as an entity.

In 1985, Beller and associates in Germany concluded from hematologic studies, renal biopsy specimens, and patient outcome that a medical disorder complicating pregnancy (e.g., liver disease, systemic lupus, was the basic problem in the HELLP syndrome).

Kirschbaum's[1] editorial comment in the 1987 *Year Book of Obstetrics and Gynecology* relating to Beller's work is most insightful: "The acronym separates out a portion of the pathology of severe preeclampsia in a way not pathologically coherant and that may be useful in the management of the disorder" (severe preeclampsia-eclampsia). Dr. Goodlin's article drives another nail in the HELLP coffin.

When children learn that Lewis Carroll's dragon, the Jabberwock, is dead they are relieved. When they learn the dragon was bogus, they are delighted. Similarly, although I am older, I am most pleased to know that the dragon HELLP syndrome was never a true obstetric entity.

Dr. Goodlin has stressed the need for a team approach to problem obstetric cases. Clearly even experienced obstetricians should confer more often when the diagnoses are questionable or obscure. Moreover, the need for long-term follow-up of hypertensive patients or those with probable metabolic disease must not be forgotten. This will further reduce material and perinatal mortality and morbidity.

Aside from the opportunity to share some of the essence of this unique study, my real reason for speaking is to applaud Dr. Goodlin for his individuality, his curiosity, his ability to ask the proper questions, and his tireless search for correct answers. He deserves special commendation also for presenting 11 papers and seven formal discussions at 13 meetings since his election to the Society in 1971, and he is a nonresident member.

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Dr. Goodlin (Closing). I thank Dr. Benson for his kind remarks. I have been privileged to speak often at our meetings, but I also lead the list in number of papers rejected by our Society for publication in the American Journal of Obstetrics and Gynecology. Whereas some of my papers accepted here had been rejected elsewhere, those rejected by the Society have often been subsequently published by other journals. My advice to younger members is to write about accepted concepts, do complicated statistics, and to remember that the difference between a rejected and a published manuscript is often the amount of postage one is willing to spend on submitting it to the various journals. Peer review does not tolerate revolution and therefore I am most grateful to the Society for accepting some of my controversial papers.

I doubt whether obstetricians will pay attention to what we say in Sun Valley about the HELLP syndrome. I fear its concepts are written in stone. About all we can hope for is that in atypical cases, caution will be used before interrupting the pregnancy.

Mechanisms of asphyxial brain damage, and possible pharmacologic interventions, in the fetus

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San Francisco, California

An examination of the cellular and molecular mechanisms of neuronal cell damage may lead to the design of pharmacologic interventions during presumed or actual fetal asphyxia. Hypoxia-ischemia in its severest form results in insufficient adenosine 5'-triphosphate production. The most important effect of this is failure of adenosine 5'-triphosphate–dependent membrane functions, which maintain ionic homeostasis, that is, ionic pumping. There is K^+ efflux and Na^+ influx across the cell membrane, depolarization of the cell membrane, opening of the voltage-dependent calcium channels, and entrance of Ca^{++} into the cell. Cytosolic Ca^{++} is also increased by Ca^{++} efflux from the mitochondria and the sarcoplasmic reticulum. Ca^{++} is a toxin in high cytosolic concentrations; it activates phospholipases A and C, which cause membrane breakdown and release of free fatty acids, including arachidonic acid. The membrane is damaged, lysis occurs, and the neuron dies. High cytosolic Ca^{++} also causes release of excitatory amino acids (especially glutamate), which overwhelm the suppressant neurotransmitters, causing seizures, increased metabolism, and aggravation of the insufficient adenosine 5'-triphosphate availability. Thromboxane A_2 is generated from arachidonic acid, increasing smooth muscle tone and thereby worsening the ischemia. Cyclooxygenase activity also results in formation of oxygen-free radicals that contribute to cell membrane damage, lysis, and death. Possibilities for pharmacologic interventions include (1) calcium channel blockers and antagonists, (2) excitatory neurotransmitter blockers, (3) oxygen-free radical scavengers (e.g., superoxide dismutase), (4) cyclooxygenase or prostaglandin synthesis inhibitors, and (5) seizure suppressants (e.g., phenobarbital). Some of these treatments have been shown experimentally to limit neuronal death in the adult and fetus, and after more investigative work they may be applicable to clinical practice. (AM J OBSTET GYNECOL 1991;164:1582-91.)

Key words: Fetal asphyxia, fetal hypoxia, fetal brain damage, fetal pharmacology

Mechanisms of brain damage

Severe fetal asphyxia may have a broad range of effects on the brain, from no evident alteration in brain function to profound cerebral palsy. In past years a large amount of information has been collected on the fetal cardiovascular and respiratory responses to oxygen limitation, giving rise to a better understanding and management of neonatal deterioration induced by asphyxia. Besides these physiologic studies, cellular and biochemical mechanisms involved in the production of brain cell death are being increasingly explored, particularly in the adult with respect to focal (e.g., stroke) and global (e.g., cardiac arrest) hypoxia-ischemia.[1] These studies have shown that complex and interrelated biochemical alterations are triggered during hypoxia-ischemia in mature subjects that ultimately result in neuronal death. Many of these alterations are also being recognized in the immature brain.[2] A thorough knowledge of these biochemical events may lead to recognition of certain steps that may be amenable to pharmacologic intervention to limit or even prevent neuronal cell damage during presumed or actual fetal asphyxia.

Current evidence suggests that, in addition to a severe hypoxic insult, some associated cardiocirculatory failure or collapse is required to produce brain damage.[3] This cardiovascular collapse probably produces a reversal of the initial cerebral vasodilatation observed in the hypoxic fetal brain, therefore adding an actual or relative ischemic component to the hypoxic insult during severe asphyxia in the fetus.

Adenosine 5'-triphosphate insufficiency. Hypoxia-ischemia in its severest form results in insufficient adenosine 5'-triphosphate production.[1, 4] Normally, up to 60% of the adenosine 5'-triphosphate consumed by the cell is used to maintain ionic homeostasis, primarily pumping sodium out of the cell and potassium into the cell. A reduction in adenosine 5'-triphosphate synthesis

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as a result of oxygen limitation therefore severely disrupts ionic equilibrium across membranes.[1-5] Potassium accumulates quickly in the extracellular space, while sodium enters the cell, carrying water with it, with the subsequent development of cellular swelling.

Other consequences derive from this ionic imbalance. First, it has been suggested that increased extracellular potassium may increase oxygen and glucose use by the brain, aggravating the adenosine 5′-triphosphate deficit. Second, extracellular potassium may stimulate glutamate-releasing neurons (see later). Third, and probably most important, as sodium enters the cell and potassium leaves it, the cell membrane depolarizes, leading to the opening of voltage-dependent calcium channels, resulting in calcium influx into the cell[5,6] (Fig. 1).

Role of calcium. Accumulation of calcium in the cytosol has been extensively demonstrated in brain cells undergoing hypoxia-ischemia.[7] This accumulation is produced through several mechanisms in addition to entry by activation of voltage-dependent channels in the plasma membrane. Calcium is also released from mitochondria that are stimulated by the increase in intracellular sodium and free fatty acids and from the endoplasmic reticulum because of the depletion of adenosine 5′-triphosphate.[8] Finally, calcium enters through agonist-dependent channels (see Excitatory amino acids).

Calcium is a toxin in high intracellular concentrations. Therefore the normal cell possesses mechanisms to maintain the free calcium concentration within tight limits.[9] These mechanisms include a calcium-sodium exchange system at the plasma membrane, an adenosine 5′-triphosphate–dependent calcium pump in the plasma membrane, a calcium-hydrogen exchange in the mitochondria, and an adenosine 5′-triphosphate–dependent uptake of calcium by the endoplasmic reticulum. The failure of these protective mechanisms during hypoxia-ischemia as a result of insufficient adenosine 5′-triphosphate contributes to calcium accumulation in the cytosol.

Consequences of accumulation of calcium in the cytosol are multiple (Fig. 2). One of the most damaging seems to be the activation of phospholipases A and C.[5] These reactions lead to membrane phospholipid hydrolysis, with the consequent disruption of cellular and organelle membranes, increase in their permeability, and further unfavorable alteration in ionic distribution. Free fatty acids, including arachidonic acid, are released from the disrupted membranes.[8] Phospholipase C also catalyzes reactions, leading to production of inositol triphosphate, a second messenger that releases calcium from the endoplasmic reticulum, and of diacylglycerol, which contributes to decreased calcium-sodium exchange.[6,8] Both reactions further augment calcium concentration in the cell and amplify its deleterious effects, thus creating a vicious cycle that ultimately destroys the cell.

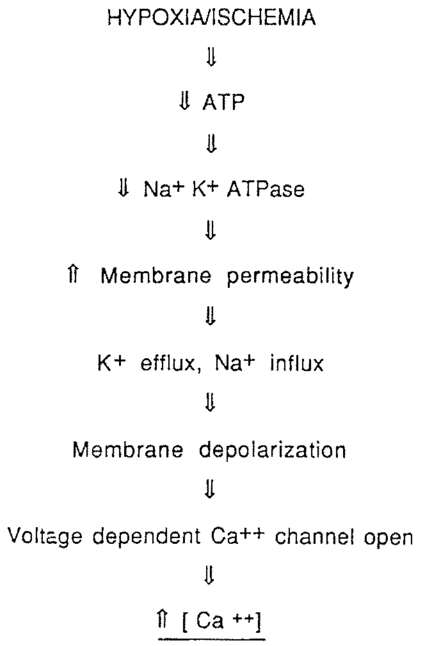

Fig. 1. Consequences of adenosine 5′-triphosphate deficiency after hypoxia-ischemia.

Excitatory amino acids. Among neurotransmitters in the brain, the amino acids glutamate and aspartate play a major role as excitatory neurotransmitters and also as neurotoxins under certain conditions.[10] Three glutamate receptor subtypes have been described,[10] designated as *N*-methyl-D-aspartate, quisqualate, and kainate receptors. These receptors are coupled to ion channels that allow sodium and calcium into the cell[9] (Fig. 3).

There are several lines of evidence that support a role of excitatory amino acids in neuronal damage during hypoxia-ischemia. First, in adults and in fetal lambs glutamate and aspartate increase in the extracellular space of the brain during hypoxia-ischemia. Second, in vivo and in vitro administration of glutamate produces neuronal death in areas of the brain with a higher density of glutamate receptors.[11] Third, glutamate receptor blockers, discussed in detail later, may limit the hypoxic cellular damage in vivo and in vitro.[12]

Neuronal cells that release glutamate are activated during hypoxia by the entrance of calcium into the cells and by the depolarization of these cells (discussed above).[10] A concomitant decrease in the adenosine 5′-triphosphate–dependent uptake of glutamate by the presynaptic membranes contributes to maintain increased extracellular concentrations of glutamate, allowing a prolonged stimulation of its receptors.

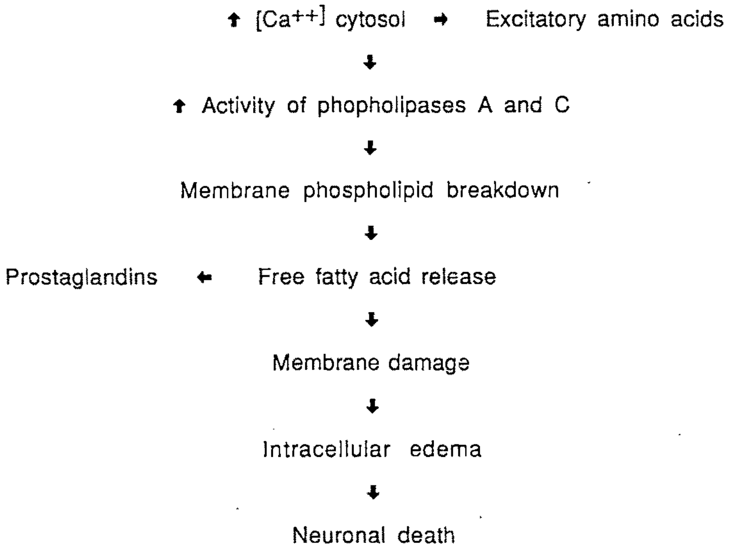
↑ [Ca++] cytosol → Excitatory amino acids
↑ Activity of phopholipases A and C
Membrane phospholipid breakdown
Prostaglandins ← Free fatty acid release
Membrane damage
Intracellular edema
Neuronal death

Fig. 2. Consequences of increased intracellular calcium.

Two mechanisms of glutamate-induced neuronal damage have been proposed.[10] The first acts within minutes after induction of the insult and involves a massive influx of sodium, and thereby of chloride and water, into the cell, resulting in osmotic load and eventual lysis of cell membranes. A more delayed mechanism of cell damage, occurring up to 24 hours after the insult, involves the influx of calcium through *N*-methyl-D-aspartate–stimulated calcium channels. The resultant increase in cytosolic calcium concentration perpetuates the process by further activating glutamate release and the other damaging mechanisms already discussed. In addition, glutamate-mediated damage of neuronal cells also results from its ability to enhance or induce seizure activity,[10] which contributes to the adenosine 5′-triphosphate depletion.

Arachidonic acid cascade. Arachidonic acid is one of the free fatty acids released from membranes by the calcium-activated phospholipases A and C.[6] Arachidonic acid initiates a cascade of biochemical reactions that lead to the production of prostaglandins, thromboxanes, and leukotrienes. In this regard both prostacyclin and thromboxane increase in the cerebrospinal fluid after cerebral ischemia in cats.[13]

Under normal conditions a balance exists between prostacyclin, which vasodilates vessels and inhibits platelet aggregation, and thromboxane A_2, which vasoconstricts vessels and promotes platelet aggregation (Fig. 4). The result of this equilibrium is an optimum blood flow in the microcirculation. However, some reactions in the prostaglandin cascade lead to production of oxygen free radicals as byproducts,[2] one of the actions of which is to inhibit prostacyclin synthetase,[14] decreasing the concentration of prostacyclin and thus leaving thromboxane A_2 unbalanced. Such imbalance promotes vasoconstriction and platelet aggregation, thereby worsening the ischemia and oxygen supply to brain cells. In addition, arachidonic acid contributes to the prolonged stimulation of glutamate receptors by blocking glutamate uptake.

Oxygen free radicals. Free radicals are molecular species with an unpaired electron in the outer orbit that renders them very reactive, with a strong tendency to initiate chain reactions that result in lipid membrane and deoxyribonucleic acid peroxidation and cell damage. Normally, >80% of the oxygen consumed by the cell is reduced by cytochrome oxidase[15] without production of oxygen free radicals. The remaining 10% to 20% follows other oxidation-reduction reactions in the cytoplasm and mitochondria that produce the superoxide anion radical.[15] To protect themselves against the deleterious effects of these oxygen free radicals, the cells possess an array of enzymatic and nonenzymatic defenses that include catalases, endoperoxidases, and dismutases among the enzymes and glutathione, cholesterol, ascorbic acid, and tocopherol among the scavengers.[1]

Oxygen free radicals are generated in the tissues during and well after the end of the hypoxic-ischemic insult (Fig. 5). During hypoxia, oxygen free radicals are formed as byproducts in the synthesis of prostaglandins by the action of the enzymes cyclooxygenase and lipoxygenase.[2, 14]

Another source is the reaction that synthesizes uric acid from adenine nucleotides.[2] As adenosine 5′-triphosphate degrades during hypoxia, adenosine

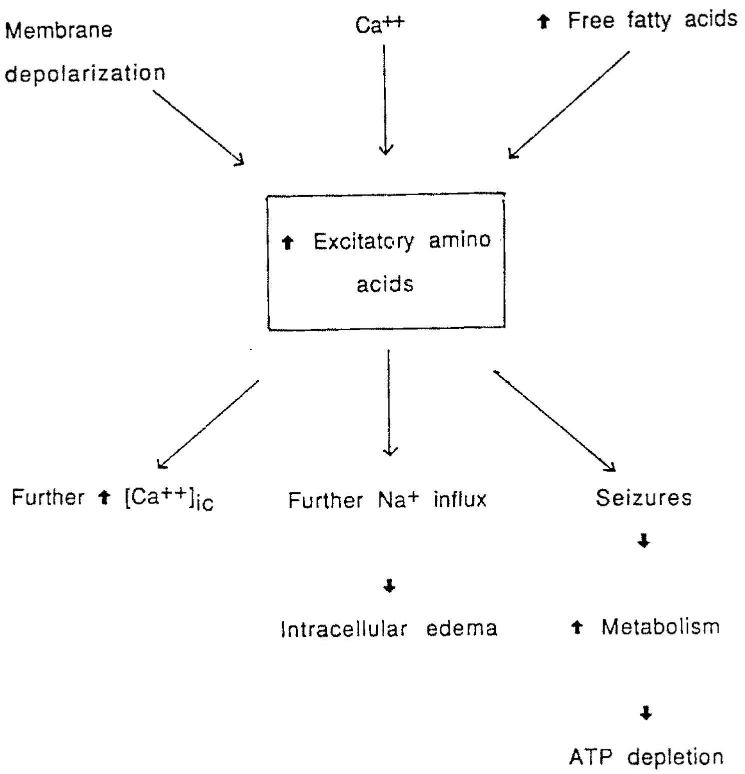

Fig. 3. Sources and consequences of increased release of excitatory amino acids.

5′-monophosphate accumulates and is not rephosphorylated to adenosine 5′-triphosphate because of the lack of oxygen. Therefore more adenosine 5′-monophosphate is converted to adenosine and then to hypoxanthine. Normally, hypoxanthine is converted sequentially to xanthine and uric acid by xanthine dehydrogenase, which uses the oxidized form of nicotinamide-adenine dinucleotide as the acceptor of electrons, without generating free radicals. However, during hypoxia, the enzyme xanthine dehydrogenase is transformed to xanthine oxidase by a reaction that is activated by the increase in the cytosolic calcium concentration. Xanthine oxidase also metabolizes hypoxanthine to uric acid, but instead of the oxidized form of nicotinamide-adenine dinucleotide, it uses oxygen as the acceptor of electrons, generating superoxide anions.

This reaction is particularly important on reintroduction of oxygen into hypoxic-ischemic tissues, which results in the massive production of oxygen free radicals that produce the so-called reperfusion injury over and above the damage already produced during the hypoxia itself. The so-formed oxygen free radicals destroy cells by peroxidation of the membrane lipids in a chain that further disrupts membranes and alters ionic equilibrium.

Summary of mechanisms. In addition to the previously discussed mechanisms, there has been considerable discussion in the literature about the role of lactacidosis, which invariably accompanies hypoxia-ischemia, as a damaging agent by itself. This controversy has been previously reviewed[1,2] and is not further addressed in this article.

The combined action of osmotic changes across the cell membrane, the catastrophic consequences of the uncontrolled accumulation by various mechanisms of calcium in the cells, the increased release of excitatory neurotransmitters, and the enzymatic and oxygen radical–mediated membrane damage overwhelm the defensive mechanisms of the cell, leading to its destruction during and after hypoxia-ischemia. Microvascular alterations produced by imbalances among opposite actions of eicosanoids further aggravate the results of hypoxia-ischemia, joining in the self-perpetuating nature of many of the reactions described above in the process of cellular death.

Strategies for intervention

From the fetal point of view, the best possible treatment for hypoxia-ischemia in utero would be removal from the unfavorable environment, which usually involves emergency delivery of the fetus. However, in

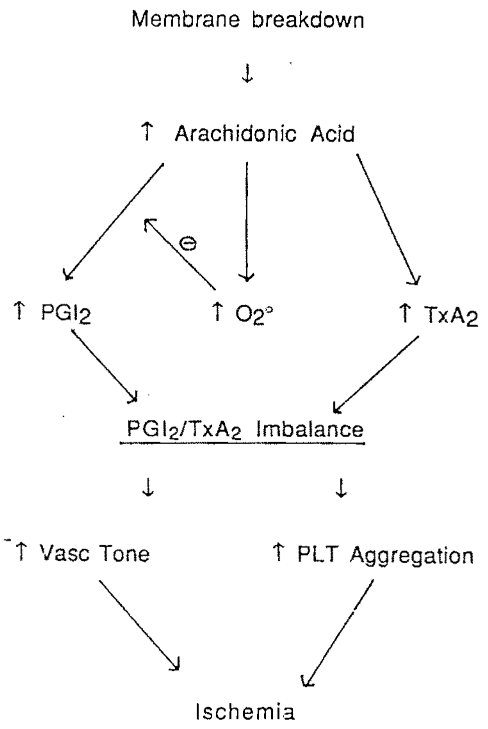

Fig. 4. Causes and consequences of prostacyclin/thromboxane imbalance.

many cases this is not always possible to achieve rapidly. Therefore pharmacologic interventions may prove to be valuable to limit or prevent damage to the brain cells until delivery and extrauterine resuscitation can occur. Such treatment should be aimed to limit the influx of calcium into the cytosol, block the glutamate receptors, inhibit the arachidonic acid cascade, scavenge oxygen free radicals, or save energy expenditure by suppressing seizures (Fig. 6).

Calcium channel blockers. It follows from the above discussion that a major intervention could be to avoid accumulation of calcium in the cytosol. Calcium accumulation cannot be completely abolished because, in the presence of continuing hypoxia-ischemia, the effects of adenosine 5′-triphosphate depletion on calcium distribution cannot be avoided. However, calcium accumulation can be limited by blocking the movements of calcium through voltage- and agonist-activated channels. If successful, these treatments could prevent the activation of enzymes and the release of excitatory amino acids induced by calcium.

Several calcium channel blockers have been experimentally tested. Nimodipine reduced infarct size and improved blood flow in experimental focal ischemia in adult rabbits.[16] Also in adults flunarizine, at a dose of 0.1 mg/kg given intravenously, decreased cell death in some regions of the brain after global asphyxia in dogs. In newborn rats subjected to hypoxia-ischemia, pretreatment with 30 mg/kg intravenous flunarizine improved behavioral scoring and provided histologic protection.[17] In newborn lambs, 3 mg/kg intravenous lidoflazine given during resuscitation after severe asphyxia was associated with improved cerebral blood flow and partial or total recovery of somatosensory evoked potentials.[18] In fetal lambs preliminary reports of the use of 45 mg flunarizine over a 2-hour infusion before total cerebral ischemia have shown a reduction in neuronal death and improvement in electrocortical activity. Flunarizine appears to be a promising agent, because it crosses the brain-blood barrier and has preferential action over cerebral circulation without other major circulatory effects.[17]

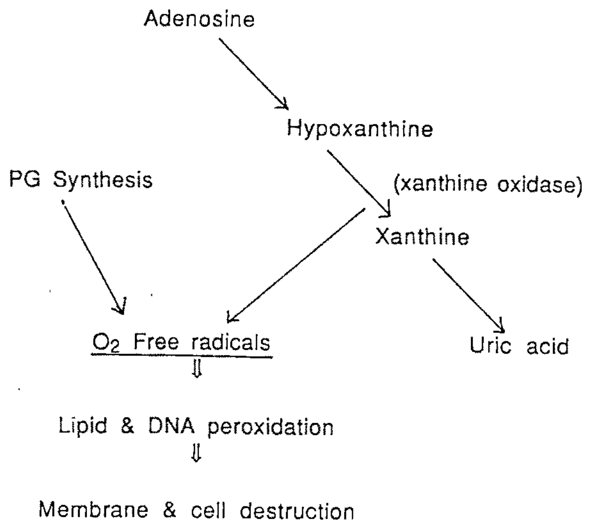

Fig. 5. Sources and consequences of increased oxygen free radicals.

A question has been raised regarding the timing of use of calcium channel blockers to obtain cerebral protection in hypoxia-ischemia. As mentioned, lidoflazine showed protective effects in neonatal sheep when given after severe asphyxia.[18] In contrast, protection with flunarizine was observed in newborn rats only when the agent was given before cerebral hypoxia-ischemia.[17] Certainly more studies are necessary to clarify this issue and others before flunarizine or other calcium channel blockers are used in the treatment of human fetuses suffering from asphyxia.

Excitatory amino acid antagonists. The deleterious actions of excessive release of glutamate during hypoxia-ischemia may be limited by blocking its binding to postsynaptic receptors with a number of antagonists that have been developed.[19] The inhibition of the *N*-methyl-D-aspartate receptor during hypoxia has been studied in vitro and in vivo with competitive antagonists such as 2-amino-7-phosphonoheptanoic acid and noncompetitive antagonists such as phencyclidine, (+)-

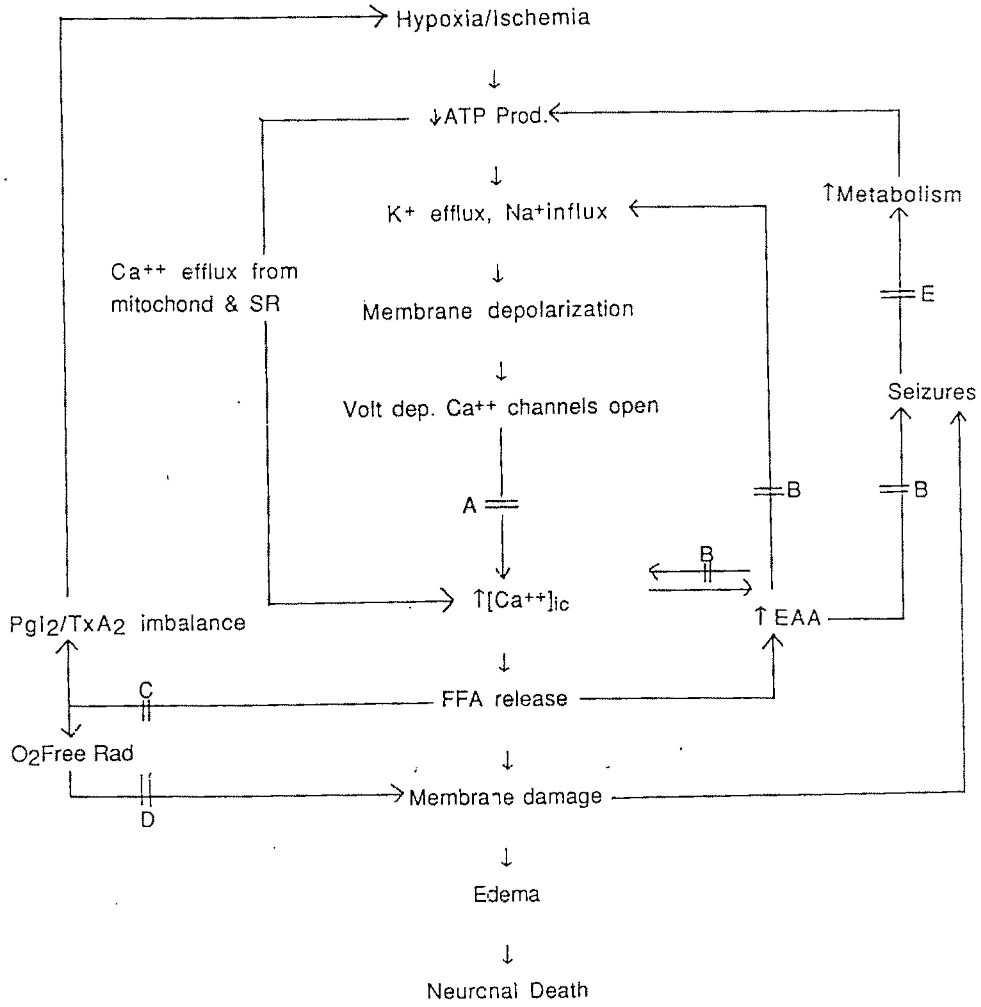

Fig. 6. Metabolic and biochemical events causing neuronal death due to oxygen limitation, and steps that are possibly amenable to pharmacologic intervention. *A*, Ca^{++} channel blockade; *B*, excitatory amino acid antagonists; *C*, arachidonic acid cascade inhibitors; *D*, oxygen free radical scavengers; *E*, seizure suppressants.

5-methyl-10,11-dihydro-^{5}H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801), magnesium, and dextromethorphan.

In adult cats subjected to complete reversible cerebral ischemia, MK-801 in doses >0.5 mg/kg given intravenously before the insult produced a return to the baseline electroencephalogram patterns on restitution of the flow, avoided secondary hypoperfusion, preserved the blood-brain barrier, and maintained the balance between prostacyclin and thromboxane in the cerebrospinal fluid.[13] In rats, local infusion of 2-amino-7-phosphonoheptanoic acid protected against the development of histologic damage induced by ischemia.[12] In newborns, MK 801 (1 mg/kg) given before, or before and after, hypoxia decreased neuronal loss in various regions of the brain in rats, and dextromethorphan (20 mg/kg given intravenously) decreased neuronal damage after ischemia in rabbits. In fetal sheep treatment with MK-801 before or up to 6 hours after cerebral ischemia seemed to abolish the appearance of postischemic seizures. The *N*-methyl-D-aspartate antagonists 2-amino-7-phosphonoheptanoic acid and MK-801 also are potent anticonvulsants. By suppression of hypoxia-induced seizures, energy can be saved and energy-dependent processes can be maintained in the brain.

Interventions to block glutamate receptors may be of particular importance in the fetus and newborn, because the brain seems to be more susceptible to glutamate-mediated damage in perinatal than in adult animals. MK-801 seems to be the most promising agent, because it is potent and crosses the blood-brain barrier. However, the potential toxicity has not been completely studied.

Arachidonic acid cascade inhibitors. Blockade of the prostaglandin synthesis cascade may limit brain cell damage by avoiding the generation of oxygen free radicals that are produced and the consequences of the imbalance between prostacyclin and thromboxane.

Prostaglandin synthesis may be blocked by inhibiting the enzyme cyclooxygenase. Among the cyclooxygenase inhibitors, the preferred drug has been indomethacin. In adult animals indomethacin given before ischemia improved postischemic reperfusion of the brain in dogs, rats, and cats.[20] During the perinatal period indomethacin has been extensively studied with regard to its actions on the closure of the ductus arteriosus and on the regulation of respiration. However, there are no studies addressing the issue of protection of the brain with indomethacin during or after hypoxia-ischemia. Until these studies are done, its value will remain speculative.

The imbalance could be corrected by giving prostacyclin. However, the results of limited studies on the use of prostacyclin during brain ischemia show little, if any, beneficial effects.[21]

Oxygen free radical scavengers. The cellular damage that results from the exaggerated formation of oxygen free radicals during hypoxia-ischemia, and especially during the reperfusion of tissues after ischemia, could be prevented by scavenging oxygen free radicals. The scavenger superoxide dismutase has been shown to improve somatosensory evoked potentials in dogs when given after spinal cord ischemia. Also, in dogs, superoxide dismutase in combination with deferoxamine, which was added to prevent membrane lipid peroxidation, improved cerebral blood flow and facilitated the recovery of somatosensory evoked potentials when it was given during resuscitation after cardiac arrest. The conjugation of superoxide dismutase with polyethylene glycol prolongs circulatory half-life and enhances superoxide dismutase entrance to the cells. This conjugate, when given before local ischemia, decreased the infarct volume in rats.[22] Catalase, an enzymatic scavenger for hydrogen peroxide, also decreased infarct volume in rats.[22] A different strategy is to block hypoxanthine oxidase, the enzyme that generates oxygen free radicals during metabolism of adenine nucleotides, with allopurinol. In rats, pretreatment with allopurinol prevented the loss of reflexes and the formation of cerebral edema after global ischemia.

Pretreatment with the combination of superoxide dismutase and catalase conjugated with polyethylene glycol improved cerebral blood flow after asphyxia in newborn lambs.[23] Also, in lambs, a mixture of three scavengers, mannitol, L-methionine, and magnesium, protected brain functions after cardiac arrest induced by asphyxia.[18] However, in this study a calcium channel blocker also was administered, making it difficult to come to a conclusion about the role of the scavengers. Finally, in immature rats pretreatment with 130 mg/kd allopurinol decreased brain edema and reduced the histologic damage induced by hypoxia-ischemia.

Despite the extensive investigations into prevention of brain and cardiac damage induced by oxygen free radicals, no clinical trials with scavengers have been done in adults,[24] and more research is still needed in the perinatal period before their use is recommended.

Seizure suppressants. Many years ago barbiturates were proposed as agents to prevent asphyxial brain damage because of their ability to reduce brain metabolism and brain oxygen consumption. Since then, most studies have shown that their protective value, if any, is rather small.[25] However, barbiturates continue to be used as a suppressant in hypoxic-ischemic seizures. By controlling seizures, energy can be saved, because it has been shown that seizures increase brain metabolic demands.[26] Therefore in theory barbiturates have a place in the prevention of brain damage during asphyxia in the fetus.

Comment

From the previous discussion, it is clear that many steps in the biochemical process that lead to cell damage are amenable to pharmacologic treatment.

In spite of the fact that most of the present information has been obtained from research in adults, the same mechanisms probably exist in the newborn and fetus. However, the relative importance of each of the mechanisms, and therefore of the steps to be blocked, has not been determined. Because of the multiple interrelations among the different mechanisms, it is possible to speculate that, after necessary extensive animal research, the strategies for treatment will most probably consist of combinations of different agents acting at different steps.

Treatment of the asphyxiated fetus has particular problems that need to be solved. The agent must cross the placenta and the blood-brain barrier without losing activity and without producing side effects in the mother and fetus. Also, the detection of impending or actual fetal asphyxia needs to be improved so that we can better decide when to administer the drugs. In this regard most of the evidence available shows that the best results are obtained when the interventions precede the asphyctic insult. This implies treating fetuses at risk and therefore that the detection of risk needs to be improved. When these and other questions are answered, pharmacologic prevention of brain damage during asphyxia in utero may be possible.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Robert C. Goodlin, Denver, Colorado. This is a significant topic. Cerebral palsy is a major disability of newborn infants. Despite our present high cesarean section rate and the use of electronic fetal monitoring, the incidence of cerebral palsy continues to increase.[1] Twenty-five years ago one of the main obstetric goals was to prevent cerebral palsy. Paul and Quilligan[2] previously estimated that 50% of cases of cerebral palsy were due to intrapartum asphyxia. Last year Naeye et al.,[3] analyzing the same collaborative project data of the 1950s and 1960s, determined that only 6% of cases of cerebral palsy are due to intrapartum asphyxia. I continue to submit an article that asks, "Have the obstetricians given up on cerebral palsy?" The reviewers not only reject it but do so very angrily. They ask what I am trying to do—reverse the concept that obstetricians are no longer responsible for cerebral palsy and attempt to treat something we can do nothing about?

My first question may be a difficult one for you, Dr. Parer. What percentage of cerebral palsy cases are due to intrapartum asphyxia? If you say it is low, then there is no clinical need for this seminar. Since there are only two to four cases of cerebral palsy per thousand deliveries, and only 6% of those are due to intrapartum factors, that means you could successfully treat only 1 in 8000 births if therapy were 100% effective. If you say it is above 10%, I can assure you, you will incur the wrath of obstetricians throughout the world.

Drug therapy for the prevention of asphyxia or asphyxial brain damage is >100 years old. My own studies were done in the 1960s, and I still believe that sedation (barbiturates), atropine, and general anesthesia offer protection for the fetus against asphyxia, although they cause lower Apgar scores. I gather that Dr. Parer disagrees with the idea that these drugs are useful.

During the past decade, fetal or newborn strokes have been diagnosed with increased frequency (about 1 in 1,000 in our hospital) with the newer imaging techniques. Would not therapy for these stroke cases of cerebral palsy be different from those cases caused by generalized asphyxia?

Neonatologists have not been able to offer effective therapy for hypoxic ischemic encephalopathy, even when the event occurs in the newborn. Measurement of hypoxanthine has not been useful, and vitamin E as an oxygen scavenger has been detrimental. Until there is an effective neonatal therapy, I doubt that there will be one that is useful for the fetus.

While reviews are useful, laboratory data are required before clinical applications of this type of intervention therapy are considered.

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DR. JAMES C. CAILLOUETTE, Pasadena, California. Dr. Parer and Dr. Goodlin each have addressed a subject that has been a perplexing one at our hospital, as illustrated by this report of a case that occurred in December 1988. A 42-year-old woman, gravida 2, para 2, at 38 weeks' gestation was attending the races at Santa Anita. For several hours she experienced severe back pain, but from the racetrack she went Christmas shopping. When she had even more severe back pain and the onset of vaginal bleeding, she went home and contacted the on-call physician. She was sent directly to the hospital and arrived within 30 minutes, at 8 cm dilatation. Electronic fetal monitoring showed late decelerations and bradycardia. She was taken directly to the delivery room, and the cervix was found to be completely dilated. Artificial rupture of membranes produced port wine fluid with meconium, typical of placental separation. A female infant, approximately term size, with Apgar scores of 2 and 2, was promptly delivered. A resuscitation team of neonatologists were present at the time of delivery and the baby was taken to the newborn intensive care unit. Twelve hours after delivery, the baby had its first and only seizure. She was given phenobarbital at that point. Computed tomographic scans, performed over the next 72 hours, showed progressive atrophy of the right parietal lobe. There has been great discussion and uncertainty on the part of the radiologist, the pediatric neurologist, the pediatrician, and the obstetrician about the pathophysiologic condition. A stroke seems possible. I hope that over time we can have a better understanding of this issue.

DR. CHARLES D. KIMBALL, Seattle, Washington. Dr. Parer, I was much impressed with your review of the molecular events linking fetal asphyxia and the transport of electrolytes across brain cell membranes. However, it seems to me you may have overlooked the profound importance on ion transport of opioid receptor ligands such as β-endorphin. Gautray et al.[1] of the Salk Institute reported several years ago in the GRAY JOURNAL that β-endorphin is elevated in the amniotic fluid in proportion to the degree of fetal distress and low Apgar scores. It is well established that the opioid receptor ligands β-endorphin and enkephalin act on nerve cell ion channels and on the second messenger adenosine 5′-triphosphate energy production. It is also clearly stated in the opioid hormone literature that the opioid antagonists naloxone and naltrexone block opioid receptors and are effective in preventing nerve cell damage and vascular shock after brain and spinal cord trauma. Opioid antagonists should have high therapeutic potential for prevention of fetal brain ischemia resulting from stress-induced hyperendorphinemia.

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DR. PARER (Closing). The incidence of cerebral palsy due to intrapartum asphyxia is, I think, <10%. Of course, an important feature of that other 90% to 95% of cerebral palsy is that by and large there isn't much we can do about it. But certainly intrapartum asphyxia leading to cerebral palsy is a potentially recognizable and avoidable cause, and therefore I think it needs to be examined thoroughly.

Dr. Goodlin, your knowledge of the literature is unparalleled in any obstetrician that I know in the world, and I must say I did not reread Claude Bernard to find his discussion of the subject 100 years ago.

One of the problems about the use of drugs to limit asphyxial damage in the past is that there hasn't been a good model by which to study it. As far as I know, the first large fetal model that we have available to study the effects of drugs on modifying damage has only been available for several years now, and that is a model that was developed in New Zealand, whereby the fetal carotid arteries are reversibly occluded.[1] One can produce brain damage in such a fetus that is quite reproducible. Unfortunately, this is almost total ischemia and thus not similar to the clinical situation. We have developed a complicated model, whereby we decrease uterine blood flow and produce hypoxia in the mother as well.[2] Unfortunately it is not as reproducible as the carotid occlusion model, but it is more like the clinical situation.

I will say nothing more about the suggestion of using atropine to abolish certain bradycardias because it has been discussed at great length in the past. I still disagree with its use.

Dr. Caillouette has presented a case to us. I do not know that the drugs I mentioned would help in such a case. However, many of us are using tocolytics such as terbutaline to decrease uterine activity while we are preparing a woman for cesarean section, so possibly these drugs could be useful as a short-term measure in a case such as that.

Dr. Kimball mentioned endogenous opioids and the possibility of blocking them. We have studied endogenous opioids in the fetus, but mainly from the point of view of their effect on blood flow. It seems that the endogenous opioids are part of a push-pull mechanism whereby blood flow to the kidney is maintained and blood flow to the muscle is balanced appropriately by the other vasoconstrictors. We have studied the use of naloxone and have some concerns about its use in the stressed fetus.[3] Of course, in the adult the idea of using the blockade is to elevate blood pressure and to maintain cardiovascular function. I do not know whether this is efficacious in the fetus.

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Ovarian response in superovulated cycles after suppression with oral contraceptive steroids

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The endocrine response to controlled ovarian hyperstimulation was reviewed in 94 women undergoing in vitro fertilization during 114 cycles. The purpose of this review was to evaluate the effect of short-term oral contraceptive suppression on the recovery of pituitary gonadotropin function and subsequent controlled ovarian hyperstimulation. Seventy-three cycles (64%) were adequate for oocyte retrieval. In 41 cycles (36%) hyperstimulation was discontinued. The serum 17β-estradiol value in women with a poor response was 57 ± 50 pg/ml on day 8 compared with 376 ± 334 pg/ml in the women who completed in vitro fertilization ($p < 0.05$). The majority of women (84.2%) had a prompt response to controlled ovarian hyperstimulation after short-term oral contraceptive suppression. Most discontinuations were due to dominant follicle selection or luteinizing hormone surge and not to oversuppression by short-term oral contraceptives. Clinical pregnancies occurred in 15 women (20.5% of harvests). (AM J OBSTET GYNECOL 1991;164:1592-6.)

Key words: Contraceptive suppression of gonadotropins, programmed ovarian hyperstimulation

Controlled ovarian hyperstimulation protocols used for in vitro fertilization (IVF) and gamete intrafallopian transfer have used clomiphene citrate and human menopausal gonadotropins. Undesirable responses to ovarian hyperstimulation have included low estradiol response, dominant follicle development, and endogenous luteinizing hormone (LH) surge.

Oral contraceptive steroids have been used successfully for pituitary suppression of endogenous gonadotropins before controlled ovarian hyperstimulation protocols for IVF.[1-3] Gonadotropin suppression before ovulation induction allows scheduling of patients at preselected start dates for ovarian stimulation and results in a more predictable follicular recruitment length and oocyte retrieval time. A potential disadvantage of contraceptive steroid suppression is a poor response to human menopausal gonadotropin therapy.[4] The poor response is presumed to be secondary to the pronounced suppression of pituitary gonadotropins. Alternatively, a failure to adequately suppress endogenous gonadotropins may result in poor follicular recruitment or the occurrence of a spontaneous LH surge.

This article reports on an experience with the use of short-term contraceptive steroid suppression before ovarian hyperstimulation and on the clinical outcomes during IVF and gamete intrafallopian transfer. Recovery of ovarian function was monitored before and during controlled ovarian hyperstimulation.

Material and methods

Ninety-four women were evaluated in a total of 114 cycles of suppression and hyperstimulation. All women were suppressed with a daily dose of 35 μg ethinyl estradiol and 0.5 mg norethindrone (Modicon, Ortho Pharmaceutical Corp., Raritan, N.J.), beginning on day 5 of the menstrual cycle preceding IVF on gamete intrafallopian transfer. The mean duration of suppression was 34 ± 2 days. Suppression continued until the predetermined start date of controlled ovarian hyperstimulation, which was accomplished with either a combination of 100 mg clomiphene citrate (Serophene, Serono Laboratories, Inc., Randolph, Mass.) and 75 IU human menopausal gonadotropins (Pergonal, Serono) or 225 IU human menopausal gonadotropins beginning day 3 after withdrawal of contraceptive steroids.

After 5 days of stimulation the follicular response was measured by serum 17β-estradiol and pelvic ultrasonography every 1 or 2 days, depending on follicular response. When the 17β-estradiol level was ≥500 pg/ml, serum samples for progesterone and LH were also measured. When the serum 17β-estradiol level was ≥750 pg/ml with at least two follicles >16 mm in mean diameter, 4000 IU human chorionic gonadotropin

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(hCG) was administered intramuscularly. Oocyte retrieval was done by laparoscopy or an ultrasonographically guided transvaginal approach approximately 34 hours after the hCG injection. Supplemental hCG (1000 IU) was administered on the day of oocyte retrieval and then every 48 hours for a total of five additional doses. A cycle was canceled if a dominant follicle was noted on ultrasonographic examination, if the 17β-estradiol level was too low (<100 pg/ml) on day 8, or if LH surge activity (a twofold rise over the previous value or a level ≥25 mIU/ml) was detected.

In 33 of the 114 cycles additional sera were obtained on cycle days 1, 3, 5, and 7. These sera were later analyzed for estrone, 17β-estradiol, androstenedione, and bioactive LH. Steroid assays were performed by standard radioimmunoassay techniques. Bioactive LH was measured by a Leydig cell assay.[5] Statistical analysis was performed with Student's *t* test, Duncan's multiple range test, and analysis of variance with StatPak (Northwest Analytical, Inc., Portland, Ore.). Stepwise discriminant analyses were done with the SPSS-X software version 2.2 (SPSS-X, Chicago).

Results

Of the 114 cycles initiated, 41 (36%) were canceled because of a poor 17β-estradiol response (18 women, 15.8%), a dominant follicle (13 women, 11.4%), or an LH surge (7 women, 6.1%). Three cycles were cancelled for miscellaneous reasons. In nine additional women an LH surge was detected in serum samples drawn just before administration of hCG (Table I).

Patient age, length of contraceptive steroid suppression, hyperstimulation protocol, and the time of hCG administration in those women who received it were compared between the group with canceled cycles and the group with retrieval cycles. There were no significant differences between these two groups, except for the difference in 17β-estradiol after 5 days of hyperstimulation, 57 ± 50 versus 376 ± 334 pg/ml (Table II).

Serum progesterone values were significantly greater ($p < 0.05$) in women in whom an LH surge was demonstrated at the time of hCG administration, 1.77 ± 0.18 versus 0.66 ± 0.08 ng/ml. There was a trend toward an elevated progesterone level in these women 14 hours earlier, 0.91 ± 0.1 versus 0.66 ± 0.08 ng/ml. Therefore progesterone was not predictive of an impending LH surge in a timely manner within a clinical setting. One of the nine women with oocyte retrieval after an LH surge did have a clinical pregnancy.

Hormone profiles of the 33-cycle subgroup that had serum sampled on days 1, 3, 5, and 7 revealed an early return of estradiol and LH after oral contraceptive suppression. Twenty-two cycles had a normal response, including six women who experienced an LH surge. The other 11 women had a low estradiol response ($n = 7$) or a dominant follicle ($n = 4$). There was a significant increase ($p < 0.05$) in 17β-estradiol and LH by day 3. There was a significant difference ($p < 0.05$) in estradiol values on day 1 in women with a dominant follicle and on day 7 in women with a low estradiol response (Table III). There were no significant differences between the normal and low estradiol responders for any of the other hormones evaluated (Table IV).

Of the 73 cycles that resulted in oocyte retrieval, 15 resulted in clinical pregnancies (20.5%). Three pregnancies occurred in women whose cycles were canceled (7.3%).

Table I. Outcome of ovarian suppression with oral contraceptive therapy

No. of cycles	114
No. of patients	94
Mean age of patients (yr)	34 ± 1
Mean duration of suppression (days)	34 ± 2
No. of cancelled cycles	41 (36%)
No. of retrievals	73 (64%)
No. of LH surges	16 (14%)
Mean duration of induction (days)	11.9 ± 0.3
Pregnancies/cancelled cycle	3/41 (7.3%)
Pregnancies/retrieval	15/73 (20.5%)

Table II. Estradiol (picograms per milliliter) after 5 days of stimulation

Low responders ($n = 18$)	57 ± 50*
Normal responders ($n = 73$)	376 ± 334
No short-term oral contraceptive suppression ($n = 5$)	313 ± 290

*Difference between other two groups, $p < 0.05$.

Comment

Suppression of the pituitary gonadal axis with oral contraceptive steroids does assist in the scheduling of oocyte retrieval in some IVF and gamete intrafallopian transfer centers. There is concern that the use of these agents can suppress the ovarian response to hyperstimulation.[4] Our data from 114 cycles with use of a low-dose oral contraceptive demonstrates that in most women the ovary responds rapidly to exogenous gonadotropins. Most of the women had a return of normal pituitary and ovarian hormonal milieu within 3 days of cessation of suppressive therapy. Sampling of serum estrone, 17β-estradiol, androstenedione, and bioactive LH obtained shortly after withdrawal of oral contraceptives was not helpful in predicting the subsequent response of ovarian hyperstimulation. Basal estrone and 17β-estradiol values were not predictive of

Table III. Estradiol (picograms per milliliter) before and during stimulation

	Day 1	*Day 3*	*Day 5*	*Day 7*
Low responders (n = 7)	8 ± 4	16 ± 4	70 ± 111	124 ± 87*
Normal responders (n = 22)	27 ± 26	98 ± 106†	311 ± 309	681 ± 516
LH surge (n = 6)	19 ± 17	104 ± 110†	289 ± 270	642 ± 355
Dominant follicle (n = 4)	281 ± 242‡	391 ± 295	698 ± 267	903 ± 585

*Difference from other groups, day 7, $p < 0.05$.
†Difference from day 1 within group, $p < 0.05$.
‡Difference from other groups by day 1, $p < 0.05$.

Table IV. Estrone (picograms per milliliter), androstenedione (nanograms per milliliter), and bioactive LH (milli–international units per milliliter) before and during stimulation

	Day 1	*Day 3*	*Day 5*	*Day 7*
Bioactive LH				
Normal responders (n = 22)	1.59 ± 0.32	2.12 ± 0.4	2.61 ± 0.59	2.2 ± 0.46
Low responders (n = 7)	1.87 ± 0.31	2.34 ± 0.81	1.62 ± 0.44	3.4 ± 1.15
Estrone				
Normal responders (n = 22)	92 ± 45	98 ± 61	151 ± 136	302 ± 287
Low responders (n = 7)	69 ± 58	31 ± 27	114 ± 84	106 ± 112
Androstenedione				
Normal responders (n = 22)	1038 ± 912	1246 ± 633	1332 ± 651	1797 ± 1176
Low responders (n = 7)	702 ± 336	876 ± 431	1206 ± 675	1445 ± 879

subsequent response to hyperstimulation protocols, except in women who had a dominant follicle. Women with a dominant follicle most likely had incomplete suppression with the oral contraceptive used in this study. Basal levels of androstenedione and bioactive LH were not predictive of poor or inappropriate response to hyperstimulation, as was previously described by Fenichel et al.[6]

In our series 36% of cycles initiated were canceled. The experience with contraceptive steroid suppression in women with canceled cycles was that the majority of canceled cycles were due not to a poor 17β-estradiol response but rather to either dominant follicle selection or an LH surge. In 16 of the 114 cycles a premature LH surge was observed and was the reason for cancelling the cycle in 7 women. Nine others had an LH surge and had an oocyte retrieval. There was one pregnancy in this group (11%) as compared with 14 pregnancies (21.9%) among the other 64 women who did not have a surge.

Despite careful monitoring of endocrine parameters, even twice a day, this study was unable to predict ovarian response to hyperstimulation or an LH surge in a timely manner in a clinical setting. Other studies have suggested that follicle-stimulating hormone before ovarian hyperstimulation may be a predictor of outcome.[7] Follicle-stimulating hormone was not evaluated in this study.

Dominant follicle selection and LH surge are not the result of pituitary suppression. A poor 17β-estradiol response may be due to the persistent suppressive effects of contraceptive steroids. This was evidently the case when a higher dose of norethindrone (1 mg) was used by Benadira et al.[4] A low-dose pill was used in the present study, 0.5 mg norethindrone and 35 μg ethinyl estradiol. The lower progestin dose may explain the difference in postpill suppression. A recent experience with a higher-dose oral contraceptive (1 mg ethynodiol diacetate and 50 μg ethinyl estradiol; Demulen, G.D. Searle & Co., Chicago) has resulted in prevention of spontaneous LH surges.[8] The women in this study had an excellent response to hyperstimulation and a low cancellation rate. Possibly a higher estrogen dose was responsible for the different experience in LH surges compared with the present study. A conception delay after contraceptive use has been reported, with a difference in fertility related to the estrogen dose of the contraceptive steroid used.[9]

Suppression with a gonadotropin-releasing hormone (GnRH) analog resulted in a more desirable ovarian response in women with a previous dominant follicle or LH surge.[10, 11] A major disadvantage has been the increased amount of menotropins required to induce an adequate follicular response.[12] Women with GnRH analog suppression require approximately 10 ampules more human menopausal gonadotropin.[13] However, if the cancellation rate is significantly <36%, it would be cost-effective to use GnRH analogs to down regulate pituitary gonadotropins in programming cycles for hyperstimulation. If pituitary suppression were prolonged after contraceptive steroid suppression, then the results should have been similar to GnRH analog suppression. This was obviously not the case with the low-estrogen-dose pills but may explain the better experience with the 50 μg ethinyl estradiol–containing pill.

Because of the high cycle cancellation rate in women with contraceptive steroid suppression, we have used 1 mg/day leuprolide acetate subcutaneously beginning 2 weeks before ovarian hyperstimulation and continuing until hCG is administered. In a preliminary group of 38 women the cancellation rate has declined to 13%, all of which was due to a poor 17β-estradiol response. Outcomes were good, resulting in a 26% clinical pregnancy rate. Others have reported the use of GnRH analogs to lower endogenous LH and have observed a higher rate of live births.[14]

The expense of leuprolide acetate and additional human menopausal gonadotropin required for ovarian hyperstimulation is prohibitive for many women. There have been varied experiences with contraceptive steroid suppression before IVF. A study comparing the performance of different doses would be worthwhile because it may be a more cost-effective method of ovarian suppression than GnRH.

In summary, the majority of women (84.2%) had a prompt return of pituitary and ovarian function after oral contraceptive therapy. A cancellation rate of 36% is not acceptable when compared with suppression with GnRH analogs (13%). However, the majority of cancellations were due to a dominant follicle or an LH surge. The cancellation rate resulting from a poor 17β-estradiol response was similar to that of leuprolide acetate, 15.8% versus 13%.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. LEON R. SPADONI, Seattle, Washington. During the past decade IVF and its progeny, gamete intrafallopian transfer, zygote intrafallopian transfer, and SOURCE (superovulation and uterine replacement of capacitated enhanced semen), to mention just a few, have captured the imagination of the medical community, infertile couples, and the media. Despite tremendous improvements in stimulation protocols, oocyte retrieval, and embryo culture, the relatively low pregnancy rate and high cost per cycle has prevented this procedure from becoming more widely available to couples who have no other avenues for achieving a pregnancy.

Dr. Burry has described a protocol for pituitary suppression of endogenous gonadotropins before controlled ovarian hyperstimulation by means of low-dose oral contraceptive steroids. The purpose of gonadotropin suppression before ovulation induction allows scheduling of patients for ovarian stimulation at preselected start times, which hopefully will result in a more predictable follicular recruitment length and oocyte retrieval time. Although this article demonstrated that short-term suppression of endogenous gonadotropins with low-dose oral contraceptives was effective in terms of a 21.9% pregnancy rate, the 36% cycle cancellation rate was found to be unacceptable. The majority of the canceled cycles were due to a dominant follicle selection, an LH surge, or a poor estrogen response. Whenever a cycle is canceled, this becomes wasted effort in terms of patient cost, time spent by the IVF team, and emotional toll on the patient. Because of the high cycle cancellation rate in patients with low-dose contraceptive steroid suppression, the authors have discontinued the described protocol and have shifted to suppression of endogenous gonadotropins with a GnRH analog. This has resulted in a more desirable ovarian response in women with a previous dominant follicle or spontaneous LH surge. Their experience with this approach has been a decrease in the cancellation rate to 13% and a clinical pregnancy rate of 26%.

Dr. Burry has appropriately brought to our attention that this results in an increased cost per cycle as a result of the increased number of ampules of menotropins that are required plus the cost of the GnRH analog. The authors should be congratulated in their attempts to find ways of decreasing cycle cost, a trend that is badly needed to help curtail the burgeoning health care costs in our society. I would encourage them to continue their research with other doses of oral contraceptives in hope of decreasing the cycle cancellation rate and still obtaining an acceptable pregnancy rate.

I would like to ask the following questions. Would you define what you mean by a dominant follicle and explain why the cycle is canceled when this occurs? Can you give us an estimate of the increased cost of using a GnRH analog for gonadotropin suppression versus oral contraceptives? With the renewed interest in using natural cycles versus controlled ovarian hyperstimulation, what impact will this have on IVF in regard to cost and pregnancy rate?

Dr. Mary Martin, San Francisco, California. You implied that using oral contraceptives might lead to a suppression of the LH surge in the following cycle. Would you clarify what you think the mechanism might be?

One of our goals since starting our IVF program has been to avoid weekend retrievals. However, using individualized monitoring produces sufficient outliers that we have been remarkably unsuccessful in meeting this goal. I wondered how successful your regimen was with regard to obtaining a predictable retrieval day.

Dr. Burry (Closing). Dr. Spadoni, a dominant follicle is one that, by ultrasonographic parameters, is mature at the first scan. If after 5 days of stimulation we saw a single follicle that was 16 to 18 mm, we considered it dominant.

The increase in cost of GnRH over oral contraceptive suppression is at least $600, often $1000. There is a significant cost increase when menopausal gonadotropins are used, an average of 10 ampules of Pergonal in addition to the cost of the leuprolide acetate, 0.5 mg subcutaneously daily (Lupron, TAP Pharmaceuticals, North Chicago).

We are doing single egg retrievals in completely natural cycles and were initially very optimistic about this method. We do start people in natural cycles who have had poor response to leuprolide and high doses of Pergonal. Our cryopreservation program, though, has been very successful. We have a 20% continuing pregnancy rate as a result of cryopreservation. Many of our couples are electing to use the more aggressive stimulation so they can take advantage of multiple cycles of embryo transfer.

Dr. Martin, I did imply a suppression of the LH surge by using a contraceptive before stimulation. That was not something that I had anticipated. It was brought to my attention in a recent article by Gonen et al.[1] I was intrigued that they had absolutely no surges in a fairly large group of patients who had suppression with Demulen, one tablet daily. In natural cycles before this regimen was used, patients had a 15% to 16% rate of LH surges, a rate consistent with most other programs. As far as mechanism, anyone's guess is as good as mine.

Our success in avoiding weekends was quite good when we were dealing with small groups of patients. Initially, we treated patients in groups of five, and it would take us a while to have five who could afford IVF. We were very successful in midweek retrievals and transfers, so that the only thing we had to do on weekends was monitor gonadotropins and do ultrasonographic examinations. Now that our program is larger, we are working more weekends.

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Significance of oligohydramnios complicating pregnancy

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Oligohydramnios is a serious complication of pregnancy that is associated with a poor perinatal outcome. Eighty pregnancies complicated by oligohydramnios constitute the basis for this retrospective study. Forty patients had premature rupture of the membranes; of these, outcomes were good in 25. Twelve of 14 fetuses with oligohydramnios and intrauterine growth retardation survived. None of the nine fetuses with severe renal anomalies lived. None of the twins with twin-twin transfusion and oligohydramnios survived. Six pregnancies with oligohydramnios and premature separation of the placenta were identified; all of these resulted in fetal or neonatal death during the second trimester. (AM J OBSTET GYNECOL 1991;164:1597-600.)

Key words: Oligohydramnios, premature rupture of membranes, renal anomalies, premature separation of the placenta

The accurate diagnosis of oligohydramnios became possible with the introduction of ultrasonographic examinations during pregnancy. Outcomes for this complication of pregnancy have been reported by several authors.[1-6] There is a consensus that severe oligohydramnios identified in the second trimester of pregnancy is an indication that perinatal outcome will be poor. Oligohydramnios may be associated with fetal anomalies, intrauterine growth retardation, and elevated perinatal mortality rates. The present paper investigates the causes for oligohydramnios and correlates the causes with outcome. A special group of second-trimester patients with both oligohydramnios and premature separation of the placenta is identified. The ultrasonographic and Doppler flow characteristics and differential diagnosis in patients with oligohydramnios and premature separation of the placenta are presented.

Material and methods

Fetal ultrasonographic studies performed at the Arizona Health Sciences Center Department of Obstetrics and Gynecology between January 1986 and June 1989 (8443 studies) were reviewed. Two-dimensional examinations were performed with 3.5 and 5 MHz transducers, and Doppler umbilical cord velocities were measured in selected patients. The diagnosis of oligohydramnios was made in 80 patients. Oligohydramnios was diagnosed when no pockets of amniotic fluid >1 cm were observed. The patients' charts were reviewed, and neonatal outcomes were recorded.

Table I. Causes of oligohydramnios

Cause		*No. of patients*	*Survived*
Premature rupture of membranes		40	25 (62%)
Intrauterine growth retardation		14	12 (84%)
Fetal renal anomalies		9	0
Renal agenesis	3		
Urethral obstruction	3		
Multicystic kidneys	3		
Premature separation of placenta		6	0
Twin-twin transfusion		3	0
Congenital anomalies (nonrenal)		2	0
Unknown		6	1 (18%)
TOTAL		80	38 (48%)

Results

The causes of oligohydramnios in our patients are summarized in Table I. The overall survival rate was 49%. Survival varied with the cause of oligohydramnios, from 84% in intrauterine growth retardation and 61% in premature rupture of the membranes to 0% in renal anomalies and premature separation of the placenta. Among those patients with premature rupture of the membranes, survival was related to gestational age at the time of rupture. At ≤25 weeks, only two of 15 babies survived (13%); after 25 weeks, 23 of 25 babies survived (92%).

The etiologic factors in growth retardation in our patients were hypertensive disorders in six, anomalies in two (Russell-Silver syndrome in 1, multiple malformations in another), and maternal drug use in two. Four patients had no identifiable risk factors. Doppler

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6/6/28665

Table II. Premature separation of the placenta and oligohydramnios

Case No.	*Age (yr)*	*Gravidity and parity*	*Onset of bleeding (wk)*	*Onset of oligohydramnios (wk)*	*Gestational age at delivery (wk)*	*Weight (gm)*	*Outcome*	*Remarks*
1	24	G3 P2	23	26	27	475	Stillborn	Doppler normal
2	35	G1 P0	10	23	27	320	Stillborn	Doppler systolic/diastolic high
3	23	G2 P1	?	20	29	1100	Death	Doppler normal
4	21	G4 P1	19	22	24	535	Death	Doppler normal
5	32	G4 P1	15	22	25	880	Death	Doppler normal
6	22	G4 P3	19	25	26	755	Stillborn	Doppler not done

G, Gravidity; *P*, parity.

umbilical cord flow studies were performed in all these patients. Systolic/diastolic ratios were high in eight, four of whom had absent diastolic flows. One fetus with absent diastolic flow was stillborn at 20 weeks' gestation. The other seven fetuses with high systolic/diastolic ratios or absent diastolic flows were delivered by cesarean section for fetal distress. Six fetuses had normal systolic/diastolic ratios; two of these were delivered by cesarean section for fetal distress.

In five of six cases, when premature separation of the placenta was diagnosed clinically and confirmed by ultrasonography, Doppler umbilical flow studies were performed. Four of the five studies were normal; one fetus had absent diastolic flow. The clinical features of the patients with oligohydramnios and premature separation of the placenta are summarized in Table II.

Comment

Causes of oligohydramnios in other studies[1,2] include spontaneous rupture of the membranes, intrauterine growth retardation, genitourinary anomalies, use of indomethacin in the treatment of premature labor[7] and abnormal karyotypes, including triploidy.[8]

The most common cause of oligohydramnios in this study is premature rupture of the membranes, and in a diagnostic plan membrane status (ruptured or intact) should be established initially. The quality of ultrasonographic examination is considerably reduced by the absence of fluid surrounding the fetus, and special care must be taken to identify fetal renal structures to exclude or establish the diagnosis of renal agenesis. Multicystic renal disease is less difficult to diagnose, because of the appearance of the kidneys and often the distended bladder on ultrasonographic examination. Oligohydramnios in twin gestation may be due to circulatory alterations, intrauterine growth retardation, or fetal death.

There have been several reports describing oligohydramnios accompanying intrauterine growth retardation. Hill et al.[9] used oligohydramnios as a screening tool for the diagnosis of intrauterine growth retardation. Philipson et al.[10] recognized that the presence of oligohydramnios should increase the clinical suspicion of growth retardation, but, if it is used as the only screening tool, 84% of cases would be missed. Steele et al.[11] suggested that four severely growth retarded infants with severe oligohydramnios had fetal renal failure. Severe oligohydramnios worsens the prognosis for survival in the growth retarded fetus; however, with active intervention 12 of 14 newborns in our series survived. Doppler umbilical flow studies with a high systolic/diastolic ratio were seen in fetuses who developed severe distress, as defined by positive contraction stress tests and fetal bradycardia. All of the viable fetuses with high ratios were delivered by cesarean section.

The early appearance of oligohydramnios in twin gestation is a very poor prognostic sign. In our three cases, all the fetuses were stillborn or died because of immaturity.

The six patients with oligohydramnios and premature separation of the placenta were of particular interest. Every attempt was made to be certain that ruptured membranes was not responsible for the oligohydramnios. Leakage of amniotic fluid could not be demonstrated in any of these patients despite multiple examinations. All patients spent several weeks in the hospital, and some received blood transfusions. All pregnancies ended in the second trimester with a uniformly lethal fetal outcome. Premature rupture of the membranes with severe oligohydramnios has been described as a risk factor for premature separation of the placenta.[12] In our cases the separation of the placenta preceded the appearance of oligohydramnios. In one study, two cases of antepartum hemorrhage were noted among 113 patients referred for fetal assessment because of oligohydramnios in the absence of ruptured membranes.[3] On that basis, active intervention for oligohydramnios when "perinatal salvage was considered feasible" was recommended. Delivery would have been inappropriate and not resulted in improved salvage in our six patients because of fetal immaturity. It is interesting to note that Doppler umbilical flow studies were normal in four of five patients with oligohydramnios and bleeding in the second trimester and were not helpful in the prediction of the adverse outcomes that occurred. This is in contrast to the outcomes in fetuses with oligohydramnios and intrauterine growth retardation. We speculate that inadequate placental function leading to inadequate fetal renal perfusion may be responsible for the oligohydramnios when premature separation of the placenta is present in the second trimester.

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Discussion

DR. RUSSELL K. LAROS, JR., San Francisco, California. Dr. Shenker and colleagues have described their experience with 80 cases of severe oligohydramnios discovered during the second trimester of pregnancy. Appropriately, they analyzed the pregnancy outcomes with respect to the cause of the oligohydramnios. Clearly, the best survival (84%) was in the 14 cases of intrauterine growth retardation. The overall survival of 62% in the 40 patients with prolonged ruptured membranes is compatible with other reports in the literature. The authors stress the importance of gestational age at the time of rupture as a predictor of outcome.

The study group includes nine patients with renal anomalies. Three of these were cases of urethral obstruction. Our genetics group has been quite successful in treating cases such as this in utero with a current survival rate of 40% in 40 cases.[1] Both catheter drainage of the fetal bladder into the amniotic sac and intrauterine surgery have been accomplished successfully. Were any of your three cases appropriate candidates for surgery?

Dr. Shenker's experience includes three cases of twin-twin transfusion with the "stuck twin" phenomenon. This is a problem exclusive to monochorionic, diamniotic pregnancies.[2, 3] The Seattle group has reported significant improvement in outcome with serial amniocentesis (31% versus 80% mortality); we have had similar results at our institution.[4]

The most significant contribution of this study is the delineation of a previously unrecognized association between chronic abruptio placentae and oligohydramnios. It will be important for the authors and others to expand the numbers of these cases to document whether the dismal prognosis observed to date holds up with larger numbers. Incidentally, my genetics colleagues tell me that they have observed persistent oligohydramnios in association with subchorionic hemorrhage after chorionic villus sampling; again the prognosis was very poor.

Finally, I would like to spend a moment reviewing a plan of action for the clinician presented with a case of oligohydramnios in the second trimester of pregnancy. The first question is, are the membranes ruptured? In general, we do not feel it necessary to attempt injection of indigo carmine under these circumstances but accept the evidence gathered by several pelvic examinations, searching for evidence of amniotic fluid leakage. If the answer is "yes," the couple should be carefully counseled about survival data on the basis of the gestational age at rupture. It is particularly important, if possible, for the couple to be fully counseled and reach a decision to terminate while the opportunity is still available, i.e., before the twenty-fourth week.

If the membranes are not ruptured, a careful ultrasonographic evaluation (searching for fetal anomalies) should be carried out. We frequently will reconstitute the amniotic fluid with an infusion of saline solution. The couple is then counseled on the basis of the prognosis for the anomaly discovered. In general, we do not do percutaneous umbilical cord sampling for chromosome analysis because the yield is quite low. Regardless of whether the membranes are ruptured, it is exceedingly important to keep the 24-week time frame in mind, so that the couple can receive complete counseling and reach their decision while termination is still possible.

I congratulate Dr. Shenker and his colleagues on a fine study and particularly look forward to seeing more data on the group with chronic abruptio placentae.

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DR. ROBERT C. GOODLIN, Denver, Colorado. Doing cesarean sections when Doppler studies are abnormal seems rather radical to me, unless diastolic flow is absent. Perhaps this was the case. Was any therapy used for oligohydramnios? In 1982 I presented some data to this society in which we used amnioinfusion and expanded the maternal plasma volume; we had cases in which the oligohydramnios was corrected.[1] In the last 2 years we have had three cases of chronic abruptio placentae, all starting before 20 weeks, and all developing oligohydramnios. We treated these patients with amnioinfusion, plasma volume expansion, and bed rest. All three went beyond 30 weeks' gestation. While the series is limited, I think there is some value in this form of therapy.

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DR. E. PAUL KIRK, Portland, Oregon. Dr. Shenker, both your article and that of Dr. Platt illustrate a problem that seems to be facing us increasingly—incidental findings in second-trimester ultrasonographic examinations. Could you help me understand the natural history of retroplacental clot or the partial placental separation found by ultrasonography in the second trimester without the presence of clinical bleeding? The relative frequency with which this incidental finding is reported presents difficulty in counseling patients.

DR. LAWRENCE D. PLATT, Los Angeles, California. What were the criteria you used to diagnose oligohydramnios? Did you use the amniotic fluid index, the 1 cm pocket, or simply gestalt? Knowing the method used is important for others to be able to reproduce this important study.

One should not be surprised that Doppler studies would be normal with placental separation. Consider why Doppler velocimetry is abnormal in intrauterine growth retardation. At the microscopic level, there is obliteration of the tertiary stem villi and smaller numbers of vessels at the 50 to 100 μm size in the tertiary villi. However, there is no evidence of these abnormalities after placental separation.

DR. SHENKER (Closing). Dr. Laros, one of our patients with urethral obstruction was referred to your department for placement of a catheter in the distended bladder. The baby, unfortunately in the group of 60% with failure, survived 10 weeks after the procedure but then was delivered in Tucson and died of pulmonary hypoplasia. One other fetus with urethral obstruction was tapped, and the fluid never re-formed in the bladder, indicating severe renal malfunction. The third fetus was not considered a candidate for surgery.

None of our patients with "stuck twin" phenomenon was treated by amniocentesis. Published results indicate that amniocentesis is a reasonable approach, a 31% survival being better than our 0%.

Dr. Goodlin, I am sorry that I did not make it clear that no cesarean sections were done because of an abnormal Doppler study. These patients had cesarean section because of fetal distress, a diagnosis based on fetal bradycardia, abnormal monitoring patterns, or positive contraction stress tests. It is only now becoming clear that the abnormal Doppler patterns are warning signs that precede the need to do anything, usually by a week or two. More data about the time of appearance of abnormal Doppler flow studies in particular groups of patients will become available. The only therapy we used for premature separation of the placenta and oligohydramnios was bed rest. Since this was not sufficient to produce any survivors, I would be interested in seeing the results of amnioinfusion in these patients.

Dr. Kirk, our two patients with premature separation of the placenta and oligohydramnios had demonstrable clots and were bleeding externally. We have seen patients with subchorionic blood collections in pregnancy with reduced or normal amniotic fluid volumes; in that case our experience has been that the prognosis is fairly good if the bleeding remains minimal and is not accompanied by severe oligohydramnios.

Dr. Platt, in defining severe oligohydramnios we used the 1 cm pocket criterion. It was essentially impossible to identify any amniotic fluid in the patients constituting this series. I am familiar with your work on trying to quantitate indices for amniotic fluid volume. We too were not surprised at the Doppler findings and included that information in this report to emphasize that the value of Doppler flow studies, as I emphasized before, is in the particular groups of patients who do have placental disease.

Hormonal inhibition of the endometrium for resectoscopic endometrial ablation

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Attempts at inhibiting the thickness and vascularity of the endometrium in preparation for endometrial ablation by using different types of hormones have been reported. To evaluate the effects of two different progestins, danazol, and leuprolide acetate on the endometrium, compared with the features of early proliferative endometrium, histologic studies were done for at least five patients treated with each hormone who then underwent resectoscopic endometrial ablation for treatment of abnormal uterine bleeding. Significant and at times dramatic differences among the treatment groups were found, with progestin-prepared endometrium being the least successful and leuprolide-prepared endometrium the most successful. In addition, the prolonged suppression provided for a period of time after the procedure by depot leuprolide is likely to help inhibit endometrial regeneration and provide even better long-term success of the procedure. (AM J OBSTET GYNECOL 1991;164:1601-8.)

Key words: Resectoscopic endometrial ablation, endometrial inhibition, endometrial histology, abnormal uterine bleeding, operative hysteroscopy

Ablation of the endometrium for management of abnormal uterine bleeding, with either a neodymium: yttrium-aluminum-garnet laser or a gynecologic resectoscope, has become a useful and important treatment option in gynecologic practice. The ideal goal is to achieve amenorrhea; however, this has been accomplished only 50% to 67% of the time.[1-4]

Because both laser and electrical energies have a limited depth of tissue penetration and thermal damage, it is obvious that attempts at inhibiting the thickness of the endometrium might improve the success rates of these procedures. To accomplish this, the use of progestins, antiestrogens (e.g., danazol, megesterol), and gonadotropin inhibitors (leuprolide, nafarelin) has been suggested.[5] Our early experience with these preparations suggested that the endometrial response to each drug grossly differed from that of the others.

This study was designed to evaluate histologically the response of the endometrium to the progestins medroxyprogesterone acetate and norethindrone, to danazol, and to a gonadotropin-releasing hormone agonist, leuprolide acetate, and to compare those effects with endometrium from days 4 to 8 of an untreated menstrual cycle.

Material and methods

Twenty-five women with abnormal uterine bleeding (excessively heavy periods with the passing of clots, gushing, or prolongation of heavy days of the period, with or without anemia) were evaluated by office hysteroscopy and directed biopsy to rule out polyps, myomas, or atypical endometrium. The median age of these patients was 43.5 years (range, 37 to 48). All were evaluated hematologically for blood dyscrasia and coagulation defects; these studies were negative. All patients had failed therapy by curettage and hormonal therapy (cyclic estrogens and/or progestins), according to a protocol approved by the investigational review board of Cedars-Sinai Medical Center, Los Angeles. None were perimenopausal or postmenopausal, none desired further fertility, and all consented to resectoscopic endometrial ablation. Preoperative preparation of the endometrium was provided with progestins (norethindrone or medroxyprogesterone acetate depot), danazol, or leuprolide acetate in a randomized fashion; five patients were included in each group. Resectoscopic endometrial ablation was performed 4 to 6 weeks after therapy was instituted in the treatment groups or on day 4 through 8 of an untreated menstrual cycle. All procedures were done with a gynecologic resectoscope (Karl Storz Endoscopy America, Inc., Los Angeles), size 24 Fr, with a 90-degree angle wire loop electrode. Electrical energy was provided by a Valleylab Force 2 electrosurgical generator (Valleylab, Inc., Boulder, Colo.), with a pure undamped (cutting) waveform at 110 W. The surgical technique involved shaving the endometrium from the cornual areas and the fundal surface down to the internal os to a depth of approximately 3 to 4 mm.

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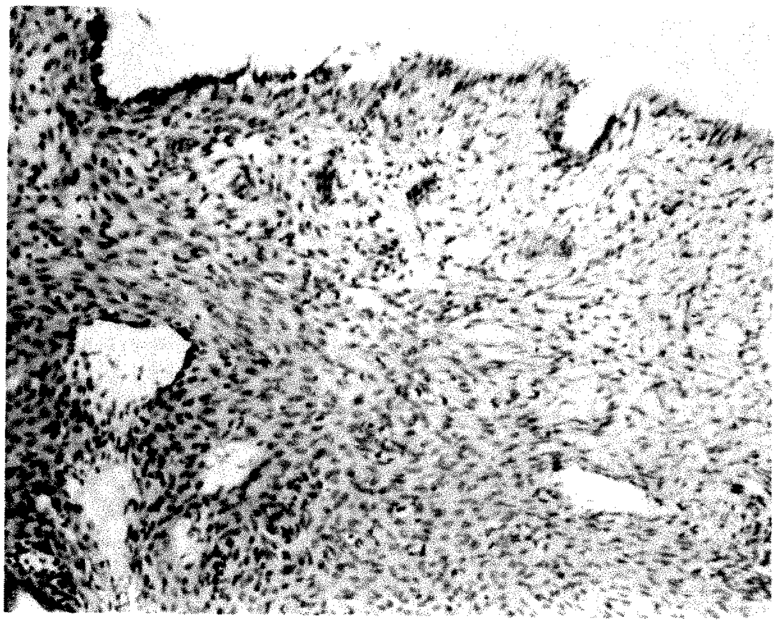

Fig. 1. Photomicrograph of endometrium after progestin therapy. Edema, angiectasia, and decidual reaction are notable.

All tissues were submitted in 10% formalin for histologic examination with hematoxylin and eosin, and special attention was given to the characteristics of the stroma, the glands, and the vessels and to the thickness of the endometrium. Some of the specimens were stained additionally with periodic acid–Schiff stain and with Masson's trichrome stain.

In addition to the above assessment, the depth of thermal damage was measured with a computerized two-point digitized method. A COHU (COHU Co., San Diego) black-and-white camera was attached to a COMPAQ DESK PRO 386/25e (COMPAQ, Houston) computer and Zeiss (Carl Zeiss Co., Thornwood, N.Y.) microscope. The program, IMAGE MEASURE 2500 IM (Microscience, Seattle), was used to assess the thermal effect on at least six representative sections of each case.

Protocols for preparation of endometrium

Progestins. Medroxyprogesterone acetate depot (Depo-Provera, The Upjohn Co., Kalamazoo), 200 mg intramuscularly, was administered for at least 6 weeks (but <9 weeks) or norethindrone acetate (Norlutate, Parke Davis, Morris Plains, N.J.), 5 mg daily, was given orally for at least 6 weeks before the ablation was performed. No additional therapy was attempted to control side effects such as breakthrough bleeding.

Danazol. Each of these patients received 600 or 800 mg (on basis of height and body weight) of danazol (Danocrine, Winthrop, New York) daily orally for at least 6 weeks before ablation procedures.

Depot leuprolide acetate. Because of the immediate gonadotropin-stimulating effect and the delayed inhibiting effect beginning approximately 10 to 14 days after injection,[6] 7.5 mg of depot leuprolide acetate (Depot Lupron, TAP Pharmaceuticals) was administered on about day 20 to 24 of an untreated menstrual cycle, and the ablation was performed between 4 and 5 weeks later.

Results

Progestin group. In a substantial number of cases the fluffy decidual character of the surface prevented adequate visualization, and often particles obstructed the outflow ports of the resectoscope sheath so that blunt or suction curettage was required before the ablation procedure was begun.

Histologically, there was diffuse edema of the endometrium. The stroma was thickened because of this edema and enlargement and deciduoid change of the cells. There was vascular proliferation and neovascularization, with the vessels having thicker and more dilated walls (angiectasia). Many areas showed increased secretory activity with the glands varying from secretory to tubular type. In none of the cases was the endometrium thin or atrophic (Fig. 1).

Danazol group. The overall suppressive effect of danazol was inconsistent. Some specimens were deciduoid, as with progestin therapy, and some were more atrophic, as with leuprolide (but not as intense), but in all cases the endometrium was relatively thick. The stroma was slightly edematous, and there was a greater variability in vascularity. Mild vascular proliferation and angiectasia were present in most specimens. The

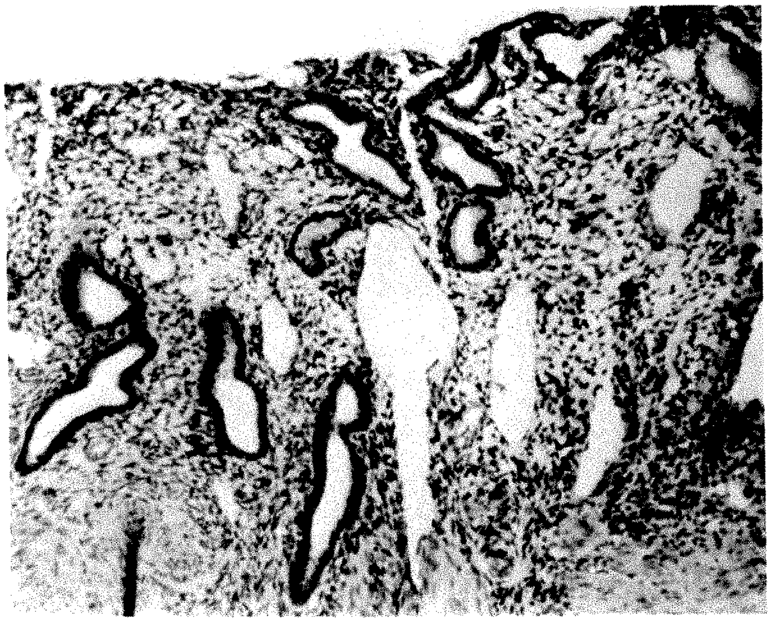

Fig. 2. Photomicrograph of endometrium after danazol therapy. Angiectasia is extensive and thickness is less than with progestins.

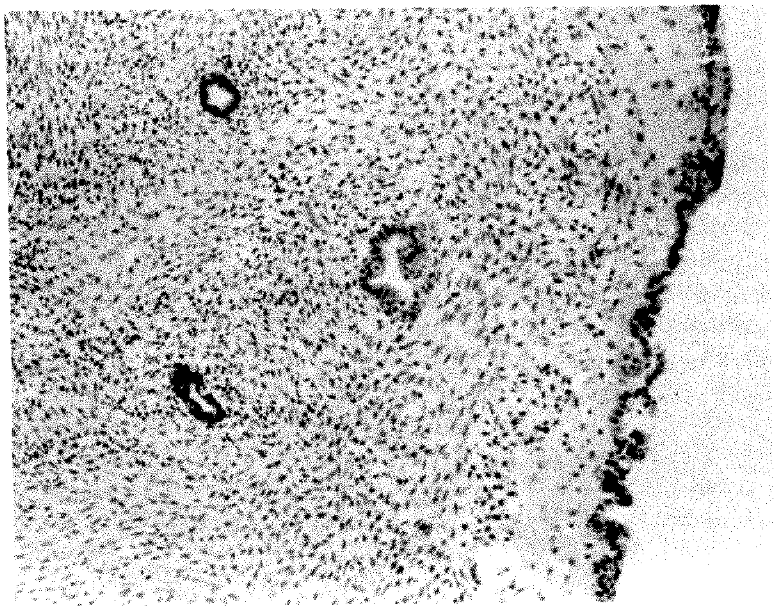

Fig. 3. Photomicrograph of endometrium after leuprolide therapy, showing atrophy, sparse glands, and dense stroma.

glands also exhibited significant variability, ranging from tubular and tortuous to mildly secretory in type but showing much dysynchrony with the stroma (Fig. 2).

Leuprolide group. In all cases the endometrium was thin and without any edema but not necessarily atrophic. The stroma had a spindle-cell-type appearance, not proliferative but more fibroblast-like, as though the cells themselves were thinned. The glands were either tubular or mildly tortuous and very reduced in numbers. Blood vessels also were reduced in number and size, but there was a slight amount of small vessel angiectasia (as contrasted to the large vessel angiectasia seen with progestin therapy) (Fig. 3).

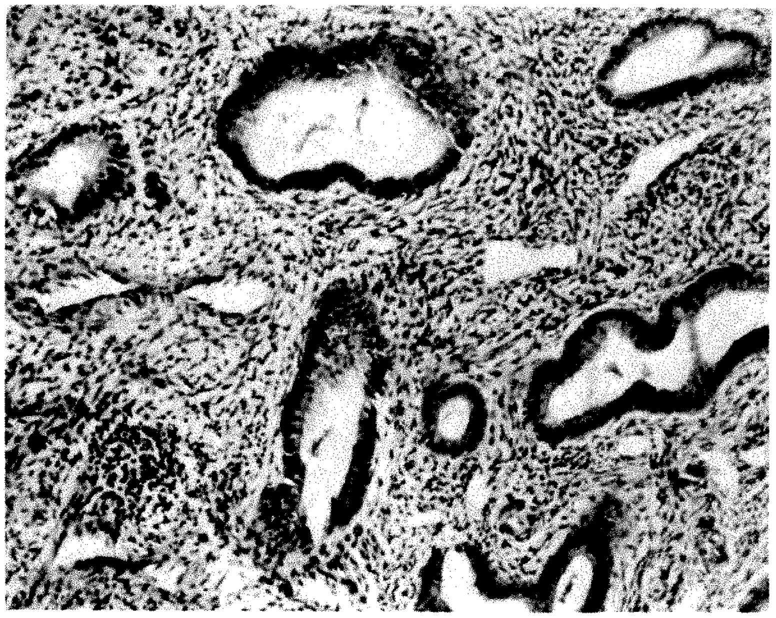

Fig. 4. Photomicrograph of endometrium on approximately day 6 of an untreated cycle, showing slight hyperplasia and mildly atypical vascularity.

Table I. Thermal damage to endometrium according to type of preparation

Type of preparation	*Mean thermal damage (μm)*	*Range of thermal damage (μm)*
Progestin	217	114-358
Danazol	175	89-300
Leuprolide	141	53-262

Untreated group, days 4 to 8. To our surprise, endometrium from the early proliferative phase in this group of patients was not the same as that seen in normal patients, as originally described by Noyes et al.[7] Although the gross appearance was that of thin, hypovascular endometrium, making the ablation very easy, histologically the tissue was dysfunctional appearing, that is, with glands and stroma being dysynchronous and with three of the specimens exhibiting a mild hyperplasia without atypia and with more spiral arterioles than expected (Fig. 4). (Is this the cause of the abnormal bleeding?)

Thermal damage. Measurements of the thermal damage revealed that the specimens pretreated with progestins had the greatest extent of thermal effect, with a mean depth of thermal damage of 217 μm, as contrasted with the mean damage in danazol-treated and leuprolide-treated tissue of 175 and 141 μm, respectively (Table I, Figs. 5 and 6).

Comment

Our experience confirms that resectoscopic endometrial ablation procedures are easier and faster when one has the luxury of time to prepare and suppress the endometrium. There is a significant difference, however, in the response of the endometrium to the different protocols that allege to suppress the endometrium.

Progestin therapy is the least successful in suppressing any of the elements studied (vessels, glands, stroma, thickness). Progestins, at least over a 6-week or 8-week period, minimally inhibit but markedly alter the endometrial histologic characteristics, with the most notable changes being the diffuse edema and vascular dilation and proliferation. It is our opinion that these changes explain why continuous progestin therapy for abnormal uterine bleeding so often fails.

Danazol works better than progestins to prepare the endometrium, but its undesirable androgenic side effects and inconsistent tissue effect, at least over the 6-week to 8-week period used in this study, make it less desirable than gonadotropin-releasing hormone agonists. Again, the breakthrough bleeding seen with danazol therapy may be explained by our observation that, in some patients, endometrial vascular angiectasia and a decidua-like reaction occurs.

Leuprolide acetate produces the most dependable suppression of all the methods tested. (Since this study was begun, this method of preparation has become our preferred method, and more than 25 additional cases have been evaluated, all with identical findings.) Although a single injection of the drug is believed not to induce castrate hormonal levels until 5 or 6 weeks after injection, our findings confirm that the early proliferative-type effect at 4 weeks after injection provides very adequate thinning of the endometrium,

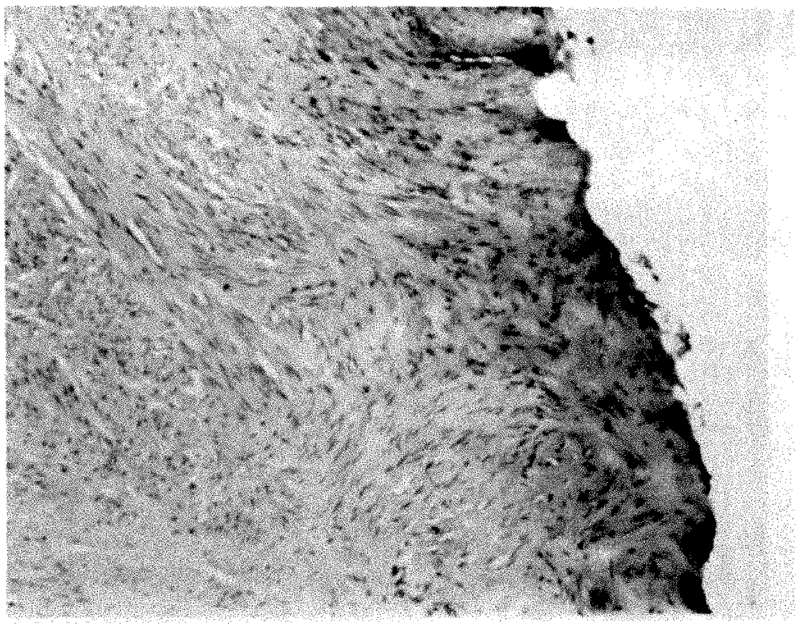

Fig. 5. Photomicrograph of endometrium showing thermal damage and the difficulty encountered in analyzing some of the tissue.

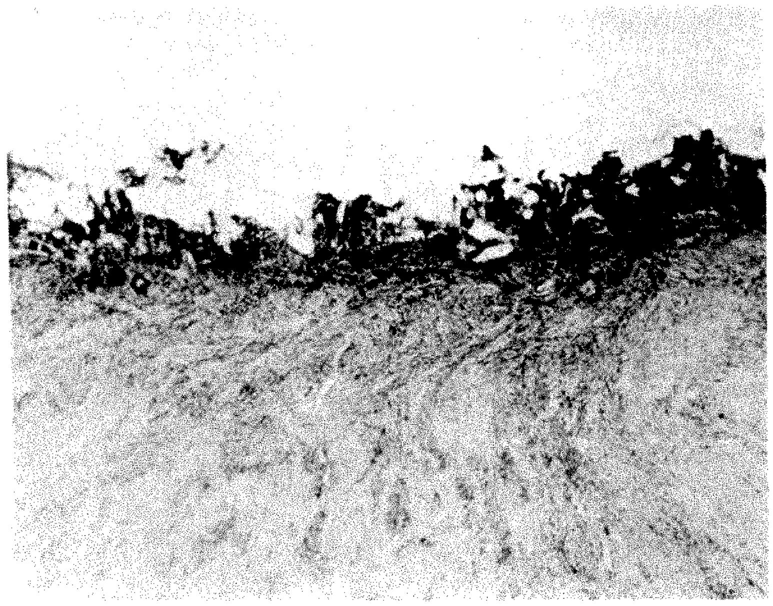

Fig. 6. Photomicrograph of endometrium after leuprolide therapy, showing extensive thermal damage.

along with inhibition of the vessels, glands, and stroma. In addition, the continual suppression that occurs for up to 2 weeks after the ablation is performed may be a very important adjunct by inhibiting regeneration of viable and responsive endometrial glands, very likely resulting in improvement in the amenorrhea rates and, ultimately, in the success of the treatment.

Performing endometrial ablations in the very early proliferative phase of the menstrual cycle is as easy and quick as in the patient prepared with gonadotropin-releasing hormone agonists. However, the lack of inhibition of endometrial regeneration after the procedure may make performing ablations during an untreated cycle less successful than in those using a preparation with a gonadotropin-releasing hormone agonist.

The exact significance of the thermal damage measurements is difficult to ascertain at this time. Of course, the knowledge of the very shallow amount of thermal damage in all of the specimens is very reassuring in that there is little risk of thermal damage to structures or organs adjacent to the uterus during these procedures. It is unclear as to why thermal damage is less in tissue with less vascularity and edema. One possible explanation is that the density and compactness of the tissue limits the depth of spread of thermal energy.

Finally, too little time has elapsed since performing these ablation procedures to comment on which method of endometrial suppression, if any, improves the amenorrhea rate the most. This will be the subject of a future study.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. David Pent, Phoenix, Arizona. The reawakening of interest in endometrial ablation, first by use of the laser and then by use of the resectoscope, has occurred within the past decade. During that time various authors have reported using a variety of medications to induce preoperative thinning of the endometrium, both to make the surgery technically less difficult and, more important, to improve the chances of success in the treatment of the patient. The two medications that have been used most frequently in the past are medroxyprogesterone and danazol, on the basis of their established track records of inducing the appropriate changes in the endometrium and endometriotic implants. The selection of which drug to use preoperatively seems to have been made on the basis of personal preference. Even in reported series that used more than one of the compounds, there was no effort to evaluate the immediate endometrial response or the long-term clinical response between the groups that had received the various preoperative medications. The one notable exception was a report earlier this year by Townsend et al.[1] In reporting on "rollerball" coagulation of the endometrium, they used three different regimens over a 4-week period to reduce endometrial thickness: danazol, ethinyl estradiol and levonorgestrel (Nordette), and medroxyprogesterone. It was thought that those patients who received danazol preoperatively had, in almost all cases, a very thin endometrium with no residual tissue. Half of the patients who received intramuscular medroxyprogesterone had a modest amount of endometrium that necessitated curettage, and histologic examination showed only a progesterone effect. Finally, the endometrium of those patients taking levonorgestrel and ethinyl estradiol was similar to the endometrium of patients who had been given medroxyprogesterone.

Dr. Brooks and his associates now present a similar study, which has been extended to include a gonadotropin-releasing hormone analog. The theoretical expectations for this compound are borne out by the histologic findings in the study, and, as Dr. Brooks indicated, that compound has become the preferred method of endometrial preparation before resectoscopic ablation of the endometrium. As he pointed out, the bottom line will be which method of endometrial suppression, regardless of the histologic picture at the time of surgery, gives the most satisfactory results with regard to amenorrhea or hypomenorrhea. In the meantime, however, it certainly is nice to have this well-designed study to provide a basis for the selection of the preoperative medication.

Dr. Brooks stated, as did almost all the others who have written on the procedure, that the "ideal goal is to achieve amenorrhea." It is true that for some patients amenorrhea is very definitely the desired end point because many of these early studies were performed on women who were extremely poor surgical risks, suffering from end-stage renal disease, leukemia, etc. In these patients total cessation of any blood loss was indeed critical. However, endometrial ablation is a procedure that appeals to women who do not want to be "cut," women who do not want a long postoperative recovery period, and those who, for various reasons, still want to preserve the uterus, including women who think that the motto of most gynecologists is "Don't take your womb to the tomb." These women, then, are seen with a complaint of hypermenorrhea, and the cure for that in their eyes does not necessarily mean amenorrhea. Some, possibly many of them, would prefer to have menstrual periods that are less in flow and duration than their normal periods had been, and so it seems that we need to recognize that hypomenorrhea might be a more meaningful end point than a surgically induced amenorrhea would be.

I would like to ask Dr. Brooks to share with us his thoughts about a concern raised by other investigators,[2] that the endometrial ablation techniques may result in cells being buried in the myometrium that later may undergo a neoplastic change and, because of their location, cannot express that change by the usual uterine bleeding. Also, Townsend et al. took some of the patients who had received medroxyprogesterone preoperatively and gave them a second injection postoperatively in the recovery room; they believed that it appeared to reduce the postoperative complaints of discharge and spotting, although they admit that the issue remains to be fully evaluated. Did you find that the use of the gonadotropin-releasing hormone agonist has resulted in any decrease in spotting and discharge postoperatively? Finally, you, along with many other authors, are concerned about the need to inhibit regeneration of viable and responsive endometrial glands to improve the amenorrhea rates and ultimately the success of the treatment. You stated in your article that this may be an important advantage of the gonadotropin-releasing hormone agonist and that the suppression continues for up to 2 weeks after ablation. Loffer[3] has questioned this role of possible endometrial regrowth, noting that there is no suggestion of any increase in menstrual flow as the postoperative period lengthens, which one would expect if regeneration were progressive. Would you please comment on that?

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Dr. Simon R. Henderson, San Francisco, California. I agree with you that there is not much difference in the gross appearance of endometrium seen in the first part of the cycle and that seen with danazol treatment. My experience is with taking down septa and removing fibroids through the hysteroscope, not with endometrial ablation. I have found use of the first part of the cycle or danazol treatment to be equally good in these patients. In using the resectoscope for removing septa in patients with recurrent abortion and infertility, I bend the wire from 90 degrees to straight to facilitate taking the septum down in the plane of the uterine cavity. I do not actually resect any tissue except where I am cutting, and I get a very nice septal excision using this technique.

Dr. Brooks, which fluid do you use to distend the uterus?

Dr. Kenneth A. Burry, Portland, Oregon. This technique is being offered to patients with perimenopausal bleeding. We now have gonadotropin-releasing hormone analogs that can cause amenorrhea and maybe better conversion of the endometrium. Dr. Brooks, do you think it is time to look at another medical approach, such as 3 months of leuprolide acetate to see what the bleeding pattern is going to be? The patients not too far from menopause may have a better bleeding pattern with just medical therapy over a short period of time until they do reach menopause.

Dr. Robert Israel, Los Angeles, California. Were there differences in the bleeding pattern and degree of abnormal bleeding among the patients in the four groups before their entry into the study? Were pretherapy endometrial biopsies performed?

Dr. Brooks (Closing). Dr. Pent, amenorrhea as the desirable goal is really something that we physicians have made up. Patients are exceedingly grateful for having lighter menstrual periods, for being able to go out of the house on those days that used to require bed rest. Our success rate in restoring menstrual periods to normal flow or better in about 140 endometrial ablations to date is about 92%. This is about the same success rate as reported for laser ablations.[1]

The question of burying cells and delaying detection of future carcinoma of the endometrium is a critical issue with which all of us who perform ablations, with either the resectoscope or the laser, have wrestled for a long time. The answer is not in yet. However, it is a general feeling that the problem is not as big as we had feared. First of all, in more than 15 years since the first ablation studies were reported, there has not been a single case of endometrial carcinoma reported in these patients. Second, to my knowledge, there has not been a single case of adenocarcinoma of the endometrium reported in patients who had previously had Asherman's syndrome. With Asherman's syndrome, not only is there obliteration of part of the cavity but there is an unresponsive, inhibited endometrium as well. Why then doesn't a uterus that has only 50% of the cavity obliterated not bleed at all with normal hormonal stimulation? I believe that an unresponsive endometrium also occurs from these ablation procedures, and this may be an environment in which adenocarcinoma might not develop.

Patients for whom this procedure fails to control bleeding all bleed externally. We know about it. The blood has access to the outside and we become aware that they are bleeding. Finally, both Goldrath[1] and I (unpublished observations) in our early experiences have found that the cavity is constricted, but not obliterated, by endometrial ablation. Goldrath has evidence from hysterosalpinography, and I have done hysteroscopy on 20 patients postoperatively. We both have found that the cavity is narrowed, more like a diethylstilbestrol cavity than like that with Asherman's syndrome. These women have a tubular endometrial cavity with a lining that consists of one cell layer of cuboidal epithelium, which is unresponsive to hormones. We believe that if bleeding is going to occur it probably will show up on the outside. Time will tell whether this is true, but I feel that hidden bleeding is something that we worry about more than we need to.

I have no evidence that one preoperative medication makes the postoperative spotting and discharge less than another. This drainage, or weeping of the endometrial tissue, is mostly a bloody discharge and not as offensive as that after cryosurgery of the cervix. I have not found that either the bleeding pattern in the first 3 months or the likelihood of resumption of periods has been substantially altered by any of the preparations. To date it is only theory that inhibition of the endometrium by the gonadotropin-releasing hormone analog for an additional couple of weeks may allow the scar tissue to predominate.

Dr. Henderson, the reason we started this study is that at hysteroscopy there is indeed a difference in the appearance of the endometrium when progestin, danazol, or leuprolide acetate is used. In fact, the increased vascularity and the friability are what prompted this investigation. Although this did not interfere with the ablation because the electrical current coagulated the vessels, I was convinced that I could distinguish a patient who had been prepared with progestin from one prepared with leuprolide acetate.

At first I, too, attempted to use the resectoscope for virtually everything. Indeed, it is easy to use. However, in treating septa and synechiae in an infertile or reproductive wastage patient, I avoid using anything that could increase scarring inside the uterus, unless it is necessary. I have gone back to using scissors since it is so simple and bloodless and scarring is minimal. By the way, you do not have to bend the wire loop since they now make a 0-degree wire loop. There is also a 0-degree pointed electrode that can be used as an incising knife.

The fluid used for all of these cases was 3% sorbitol, although glycine seems to be just as effective. Others have reported the use of Hyskon. I choose not to use Hyskon because I do not like the pictures I obtain through this medium nor do I like the stickiness and the need to rinse my equipment. Sorbitol, in a continuous flow system, is a very effective and useful medium and allows for the use of electrical energy under water.

Dr. Burry, endometrial ablation was done in some patients who cannot wait very long. Some of our patients do not go into the menopausal state until age 55 these days. I would be concerned about the safety of gonadotropin-releasing hormone analogs used, even intermittently, for such long periods of time. I do not believe there are good data yet on this question. However, it may very well be useful to try 3 months of a gonadotropin-releasing hormone analog and watch the bleeding pattern.

Dr. Israel, the bleeding patterns were fairly similar. All patients had previous dilation and curettage or hormones as treatment of excessive bleeding. We were on a very strict protocol at Cedars-Sinai because the use of the resectoscope was considered investigational. All patients had previously been treated for excessive bleeding. All had office hysteroscopy performed before the endometrial ablation to rule out myomas, polyps, or atypical endometrium.

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Vulvar vestibulitis: Prevalence and historic features in a general gynecologic practice population

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Portland, Oregon

All gynecologic patients seen by the author during a 6-month period were questioned and examined by means of a swab test to determine the prevalence of vulvar vestibulitis and the normal variation in sensitivity of vestibular skin. Of 210 patients, 78 (37%) had some degree of positive testing. A total of 31 patients (15%) were found to fulfill the definition of vulvar vestibulitis. A questionnaire was administered to these patients as well as to seven patients in whom vestibulitis had been previously diagnosed. A total of 50% had always had pain, most since their teenage years. Their history was not suggestive of a cyclic or remittent pattern of symptoms. Those with secondary dyspareunia or resolution of pain were usually either in a post partum phase or had group B streptococcus or human papillomavirus. The two most severe cases of vestibulitis occurred after use of fluorouicil cream. A total of 32% had some female relative with dyspareunia or tampon intolerance, raising the issue of a genetic predisposition. (AM J OBSTET GYNECOL 1991;164:1609-16.)

Key words: Vulvar vestibulitis, dyspareunia, focal vulvitis

Vestibulitis is the name given to the problem experienced by women with painful introital skin in whom clinical confirmation is by demonstration of pain with light touch of a cotton-tipped swab. It has been described in recent medical literature as well as in old references, but there is no agreement as to cause.[1, 2] Prevalence data have not been published previously. This article attempts to establish prevalence in a general gynecologic practice as well as to define the spectrum of sensitivity of the vulvar vestibule. To elucidate symptoms and history, patients with vestibulitis were interviewed.

Methods

All gynecologic patients seen by the author in her private practice between July 1, 1989, and Dec. 31, 1989, comprise the study group. Patients were not included if they did not require a pelvic examination, were less than 3 months post partum, or were currently pregnant, although patients seen for follow-up 2 weeks after a first-trimester miscarriage or termination were included. Other patients excluded were those distraught for some reason (1), those inadvertently not questioned (2), and one 80-year-old patient whose status was post radiation and vaginectomy. Those patients who had repeat visits during the study period were entered only on the first visit.

Each study patient was asked the following question: "Do you notice now, or have there been times in the past when you have had pain at the opening of the vagina either in inserting a tampon or with sexual activity?"

Information was then gathered about each patient noting race, parity, and results of the swab test. The swab test was conducted at the sites noted in Fig. 1 with a water-moistened cotton-tipped swab. For reference, patients were asked whether touch at an adjacent site felt normal and not painful and were then asked to compare sensation at six points. The swab was rolled over the skin gently at each site, avoiding rubbing. Five grades of response were considered: 0, normal sensation; 1, unpleasant, scratchy sensation; 2, painful, sharp sensation; 3, patient acknowledged pain and was noted to flinch; 4, patient noted significant pain and recoiled. Patients' subjective answers provided the means for differentiating grades 0 to 2. The sites chosen for testing were intended to be representative but not inclusive.

A questionnaire was administered by telephone or in person by the author to survey those who were found to have vestibulitis during the study period. To this group were added the data from the same questionnaire administered to seven other patients in whom vestibulitis was diagnosed by the author before the study.

Vestibulitis was defined as the condition characterized by entry dyspareunia, pain experienced when inserting tampons, or pain with speculum insertion, cou-

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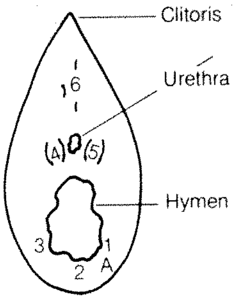

Fig. 1. Swab test sites: a comparative site for reference *(A)* and six sequential test sites.

pled with sensitivity to touch with a swab in an aforementioned area of the vulvar vestibule. Borderline vestibulitis was a term used for the subjects who fulfilled the above criteria but denied having had to adjust sexual activity or tampon use.

Statistical significance of categoric variables was assessed with χ^2 analysis.

Results

During the 6-month study period 210 patients were entered in the study. All were white except for four who were black, and one each who was Native American, Hispanic, Chinese, and Japanese. Of those with vestibulitis, all were white except one patient who was Japanese and one Chinese patient who had borderline vestibulitis. Age distribution in the study group was 11 to 80 years.

Questioning revealed 43 subjects (20%) with symptoms of pain. Of these, all except three had positive swab tests, fulfilling the definition of either borderline vestibulitis (4%) or clinical vestibulitis (15% of the whole group) (Fig. 2). The three with negative swab testing had, in one case, a diagnosis of vaginismus after early sexual abuse, and, in two cases, no physical explanation for their complaints.

Of the remaining 167 subjects with no pain, 38 had positive swab test results at at least one site.

The likelihood of sensitivity at each site is compared in Fig. 3 for the group with vestibulitis. Patients with borderline vestibulitis or without symptoms had relatively lower percentages of positive sites but the order of sites likely to be tender remained the same: the area near each Bartholin's duct, the crypts adjacent to the urethra, the midline of the posterior fourchette, and finally the area of anterior epithelium between the urethra and clitoris. The degree of tenderness followed the same relative distribution as did the site prevalence, with most tenderness occurring in the paramedian rather than midline sites and more often posterior than anterior. In only three women were the paraurethral and anterior sites the most tender.

To explore what distinguished each subgroup, the data were separated into number of positive swab sites per patient and grade of most sensitive site per patient (Fig. 4). There was overlap between groups, and, although statistical significance was not achieved, the borderline group seemed distinguished from those without symptoms not by the severity of the worst site, but by number of positive sites. The difference between the vestibulitis group and women without symptoms was significant; 50% had five or six positive sites and had pain significant enough to cause flinching or recoil in reaction to the light swab touch.

Relationship to parity. The proportion of nullipara to parous women in the combined vestibulitis categories was significantly greater (55%) than that in the nonvestibulitis subjects (38% $p < 0.05$). More details emerged from the interviews.

In an attempt to clarify whether vestibulitis has a significant tendency to be episodic, answers were tabulated to the question of subjects having had previous vestibular pain. Of all subjects with no present complaint but recalling previous pain (25), four were nullipara, and only two described what may have been vestibulitis. Of the 21 parous subjects, all except five specified that the previous pain had been post partum rather than interval. Two of those five had had previous vestibulitis confirmed by the investigator. Thus, only four of 167 subjects (2%) without present vestibulitis had had prior vestibulitis excluding instances post partum, suggesting that aside from that circumstance it is not a recurring syndrome.

A history of having had post partum dyspareunia was associated with no greater likelihood of having a positive swab test; however, patients in whom post partum vestibulitis developed had a risk that it would become chronic, as shown by interview data.

Interview results of 38 subjects. Vestibulitis was diagnosed in 31 patients during the 6-month study period. This group was interviewed as were seven other patients in whom vestibulitis was diagnosed before the six months. Table I shows results of these interviews. The median age of subjects was 34 years and the median length of complaint was 8.5 years. If three patients are removed from the calculation who were known to have had postpartum vestibulitis resolve at 0.3, 0.9, and 2 years, the median length of complaint is 10 years. Half the respondents first noted pain on trying to use tampons, rather than during sexual activity. Twenty-eight women either avoid or restrict tampon use, and 14 noted that removal hurts more than insertion. Five patients described pain with sex but said they can use tampons without discomfort; only one has the converse situation.

Respondents were asked about their sexual relationships. Many admitted that they have reduced sexual activity because of pain unless their partner is female,

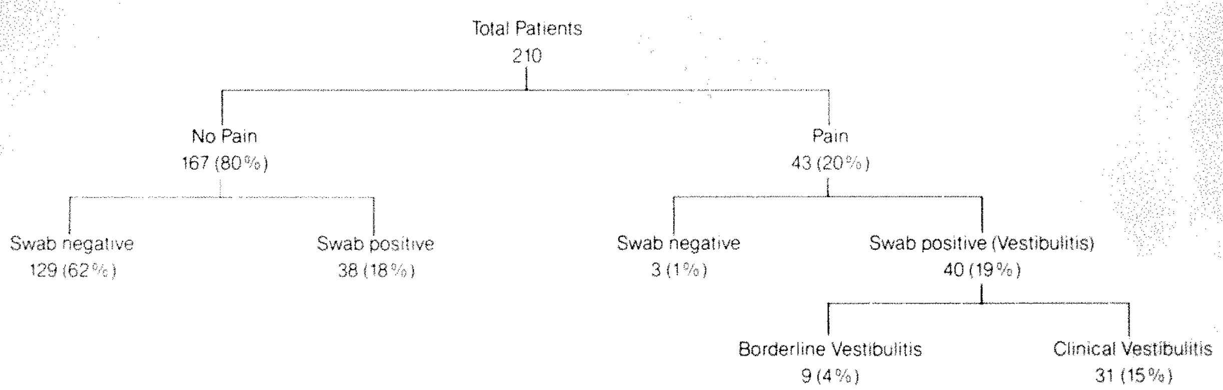

Fig. 2. Study population as it became segregated by categories of present complaints of pain and cotton swab testing.

but more than half thought that they and their partner had either come to an accomodation or that the problem was so minor that their relationship was not disturbed. At the other end of the spectrum, however, several subjects attributed failed relationships or significant stress to having vestibular pain. Often subjects wondered whether they were unique or had a hidden emotional aversion to sex. Even if they did not believe that the problem required significant intervention, a diagnosis and explanation helped both partners. It is noteworthy that half of the respondents had consulted a doctor; 37% had been told or had assumed that they were probably suffering from yeast infection.

The group was almost evenly split between those whose history was consistent with primary versus secondary vestibulitis. There was no difference in the amount of present pain and level of dysfunction described by those with primary as opposed to secondary vestibulitis. However, the average level of worst pain recalled in the past was greater for those with primary vestibulitis, usually being described as so severe as to make sexual relations impossible or even to cause continuous vulvar pain, whereas those with secondary development of sensitivity usually had continued some sexual activity even at their worst.

Of the 65% of subjects who noted variation in symptoms over time, most recalled gradual changes over months or years. The usual trend was that patients had lessening pain over time, although there were five with symptoms that were worsening; two of the five were perimenopausal and not taking estrogen. Thirteen other subjects had unchanging symptoms.

Obstetric history was noted, especially because there was a significant subgroup of patients (21%) with vestibulitis that started post partum and had not resolved. The entity known as postpartum dyspareunia fulfills the diagnostic criteria of vestibulitis. Vestibular pain was recalled by all patients delivered of infants by cesarean section. Post partum was commonly recalled as the time of worst vulvar pain by those with vestibulitis, but among multipara more than half noted relative differences, sometimes dramatic, in vestibular pain after different deliveries.

Histories of prior infection with human papillomavirus (HPV) are summarized in Table I and indicate that a majority of subjects with vestibulitis had no known association with this virus. The most significant correlation that could be found was that in patients with a positive HPV history, vestibulitis was much more likely to be a secondary condition rather than primary ($p < 0.05$). Colposcopic examinations were performed in only eight patients as dictated by severity because it was beyond the scope of the study to test routinely. Colposcopic evidence of HPV could only be found in one patient at the site of swab testing and she had had recent symptom resolution. Two patients who underwent surgical revision of the posterior vestibule did not have histologic evidence of HPV. Ironically, treatment for HPV with unusually large doses of fluoroucil cream (daily and immediately after lasing) had caused severe chemical burns in two patients and evolved into the most severe cases of vestibulitis seen in the study. One of those patients found that symptoms improved over 2½ years with an interval pregnancy, delivery, and lactation. The other had no improvement with trials of topical estrogen, locally injected dexamethasone, and topical lidocaine jelly over 1 year so underwent surgery with good although not complete relief. Two other patients had used fluoroucil cream without any affect on the vestibulitis.

The only infectious agent found to directly cause or worsen vestibulitis was group B streptococcus, noted in two patients. Although it was necessary to treat repeatedly, there was full resolution in one subject after 2 years of dyspareunia and reduction of pain in the other subject. Other patients who noted resolution were one with HPV cited in this article, one whose symptoms developed in the perimenopause and later resolved,

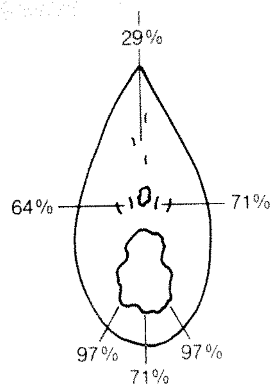

Fig. 3. Percentage of vestibulitis subjects positive at each swab test site (n = 31). All subjects tested positive at more than one site.

Table I. Interview results in 38 patients with vulvar vestibulitis

Feature	*No.*	*%*
No. of patients	38	—
Median age at study (yr) (range)	34 (11-50)	—
Median age at discovery (yr) (range)	21 (11-48)	—
Median length of complaint	8.5 (0-34)	—
(yr) (range) excluding 3 post partum who resolved	10 (0-34)	—
Sexual activity		
Active with male partner	25	66
Pain inhibits activity	Yes-14 No-11	—
Disturbs relationship	Yes-12 No-13	—
Active with female partner	6	16
Pain inhibits activity	Yes-1 No-5	—
Disturbs relationship	Yes-2 No-4	—
Inactive	4	10
Because of pain	Yes-2 No-2	—
It disturbed relationships	Yes-2 No-2	—
Never sexually active	3	8
Pain a deterrent	— No-3	—
History of pain with sexual activity		
Has always been painful	Yes-16 No-19	46/54
HPV disease		
Condyloma or dysplasia treated in past	11 of 38	29
Known previous vulvar condylomata	7	18
Papanicolaou smears always normal	23	61
Examined by colposcope	8	—
Positive white epithelium on vulva	2	—
Positive at swab site	1	—

Table II. Vestibulitis categories

Primary	*Secondary (acquired)*
Greater proportion of nullipara Histories of worse pain Family histories strongly positive	Often post partum onset Associated with group B streptococcus, high-dose fluorouracil cream, and human papillomavirus history Negative association in sisters

and three postpartum patients who had resolution while under observation after having had dyspareunia for 2 years, 11 months, and 4 months, respectively. Hormone status did not explain the continuation or the timing of resolution of vestibulitis in most postpartum patients.

One third of respondents knew of a female relative with dyspareunia or intolerance of tampons. Of those with sisters, this association was quite strong for those with primary vestibulitis (8 of 10) and not found among women with secondary vestibulitis. One subject asked each of 12 female cousins at a family reunion and found that all note tampon pain. Another patient deduced from family comments that there were four generations of women on her maternal side who had probably been affected.

Subjects were asked about possible autoimmune problems and one each had the following: Sjogren's and Raynaud's phenomenon, temporal arteritis, positive results from the Venereal Disease Research Laboratory with negative workup, and thyroid disease.

Two patients with vestibulitis were exposed to diethylstilbestrol, one with very extensive vaginal adenosis.

Comment

An entity fitting the description of vulvar vestibulitis was described a century ago according to Friedrich,[3] and was noted in textbooks of gynecology until the 1930s. References then disappeared from the literature until the 1980s when several reports reestablished its existence. In contrast to reports derived from referral practices of women who had significant complaints, this cross-sectional study of a general private practice elicited information which had usually not been volunteered by the subjects and revealed the prevalence of vestibulitis to be 15%. That such a high proportion of study subjects was found to fulfill the definition of vestibulitis was surprising. However, the swab testing also revealed that vestibular sensitivity describes a continuum from those with swab sensitivity but no pain, to those with tolerable borderline sensitivity, to those with significant dysfunction. It is important that the problem of pain was minor for many, and accomodation was aided even by getting an explanation of the problem. It nevertheless reemphasizes that sexual dysfunction is often underreported and underdiagnosed, indicating the importance of an astute clinician who feels comfortable routinely asking about sexual concerns.

Subjects in this study had a long median length of involvement, presenting a picture of sensitivity that predated sexual exposure in many cases, although data for that was not specific. That so many subjects noted problems in their teenage years raises the question of

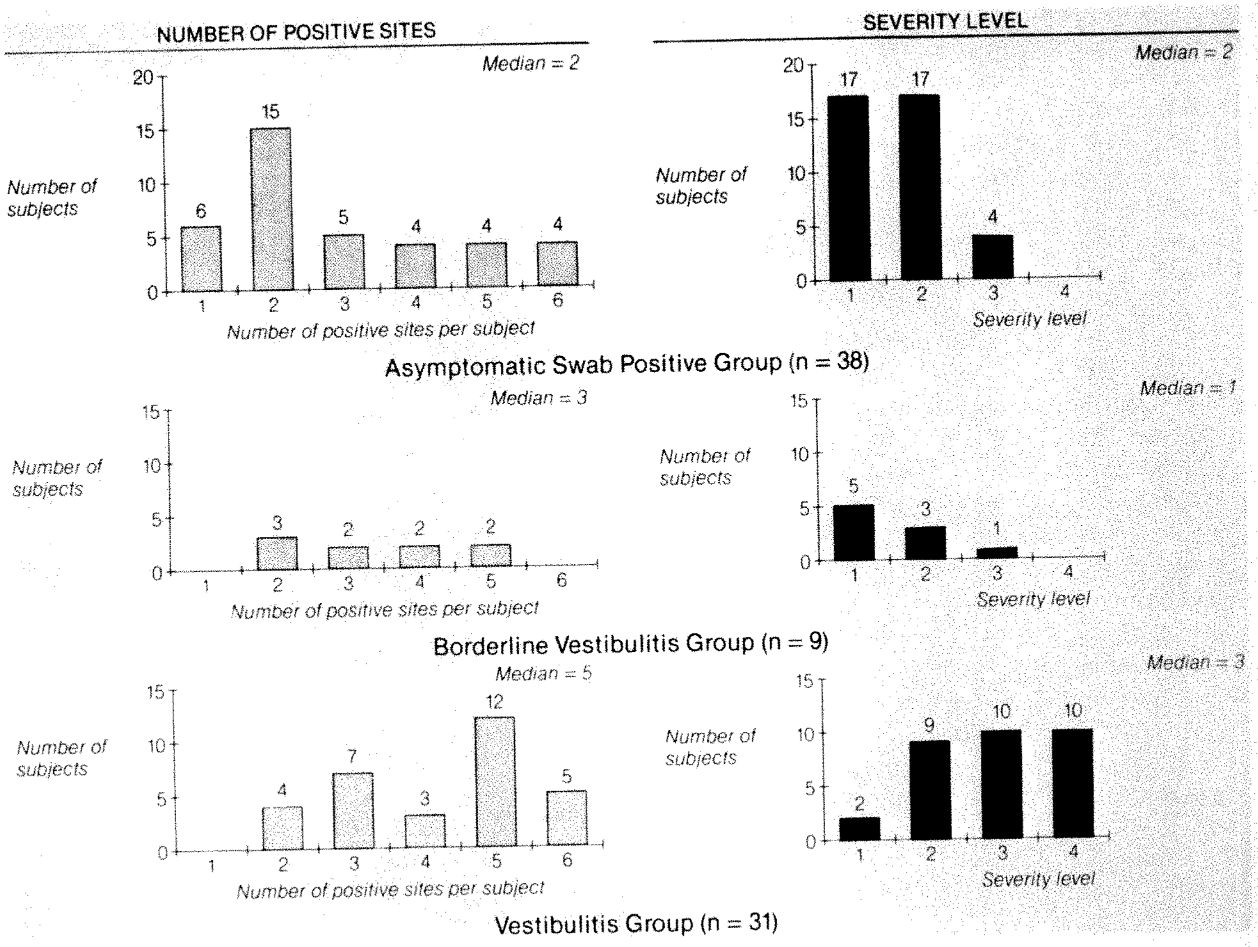

Fig. 4. Paired charts for each group illustrate (at left) the number of positive swab sites per subject and (at right) the severity of the most painful site per subject. Severity levels: 1, scratchy; 2, sharp; 3, evokes flinch; 4, evokes recoil. Statistical significance was present in comparing medians of subjects with vestibulitis with women without symptoms both for worst site ($p < 0.001$) and number of sites ($p < 0.001$).

there being a bimodal occurrence of vestibulitis (Table II), one group having it from early development and the other group, perhaps arising from those with greater sensitivity but no symptoms, noting it after an aggravating factor such as peripartum hormone changes or HPV infection. The data gathered here indicate that vestibulitis develops only in certain women when they are exposed to these factors. Some of the discrepancies in the literature with regard to HPV involvement[1-6] may be explained by this theory of acquired versus primary complaints. Woodruff and Parmley[7] reported that 27% of their group dated the onset of symptoms to the postpartum period, as did 21% in this study. Dyspareunia after delivery has not been identified previously as vestibulitis but fully satisfies the definition. A longitudinal study of postpartum patients is under way by the author.

Contrary to Peckham's findings,[2] spontaneous remissions were rare among patients in this study unless the vestibulitis had developed post partum. Were there to be a remission rate of 50% as Peckham suggested, the high prevalence rate found in this study would lead to the expectation of finding a large number of women who recalled past pain that had spontaneously resolved. This was not the case. The large proportion of subjects with primary vestibulitis in this study (50% in contrast to Peckham's 9%), strengthens the hypothesis that there is an inherent variation in sensitivity of the vulvar vestibule and that women may have vestibulitis with no antecedent irritant. The vestibule is the only genital tissue derived from endoderm[8] and may have unique developmental features.

Questions for future study would include clarification of any relationship such developmental influences as diethylstilbestrol or genetic markers such as HLA type might have to vestibulitis. Several findings that were anecdotal in this study should be investigated, such as whether family members really have vestibulitis by examination and whether a history of childhood sexual abuse influences later complaints. More studies are needed that test for unequivocal evidence of HPV and follow up results of topical or laser therapy. This may be particularly important in patients with acquired vestibulitis.

Unlike other studies, this one documented many patients with mild-to-moderate complaints. Treatment suggestions for most included continued use of artificial lubricant or use of 2% lidocaine jelly at tender sites before intercourse. Adequacy of 2% lidocaine therapy was not fully assessed. It could be that 5% lidocaine solution is more satisfactory in some patients. Surgery should be reserved for those with severe vestibulitis; other researchers have reported high rates of improvement or cure.[9]

Because the two worst cases of vestibulitis seen in this study occurred after greater than average use of fluorouracil cream, caution is warranted especially with regard to use after extensive lasing or without sufficient intervals between applications to allow recognition of chemical injury.

In conclusion, vestibulitis was found to have a prevalence of 15% in this general gynecologic population. Affected women could be divided by primary and secondary involvement, with postpartum factors, chemical burns, group B streptococcus, and HPV being implicated in secondary development of the syndrome. Half the subjects had adjusted satisfactorily to the discomfort, but vestibulitis remains an underdiagnosed problem with a confusing natural history.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. CHARLES BROBERG, Pasadena, California. The patient with a problem of vulvar burning, itching, irritation, and dyspareunia has been the source of great frustration for me for years. I suspect that most of us have had more than one such patient who has left our offices as uncomfortable as when she entered, probably to seek a solution from another gynecologist. We followed our best judgment and tried many treatments. When we discovered an etiologic agent, the choice of treatment has been clear, but when no definite cause was found, our treatments tended to be empiric and often rather creative. Success has been inconsistent enough that no clearly best method has emerged.

Dr. Goetsch has undertaken a study of patients from her practice to determine the prevalence of the entity known a vulvar vestibulitis syndrome and to provide some standards for diagnosis and assessment of severity. A prevalence of 15% seems high for my practice, but perhaps if I asked more pointed questions or used the Q-tip more frequently I would discover I have been dealing only with the tip of the iceberg all these years. Because the literature has only recently begun to discuss this problem, she assures us that we are not alone in our frustration. It is reported that symptoms often improve or resolve completely with minimal or no treatment.[1] I hope this article will stimulate some good minds to discover a true cause and to suggest a reasonable therapy that will relieve those who suffer from persisting symptoms.

The term *vestibulitis* is of recent origin, but reports of various conditions of the vulva associated with pain or dyspareunia are not. In 1976 the term *erythematous vulvitis en plaques* appeared in the literature.[2] In 1983, obstruction of the Bartholin duct as a cause of pain with sexual activity was described.[3] The term *vestibular adenitis* attributed the symptoms to inflammation of minor vestibular glands.[4] The use of a cotton-tipped applicator to aid in diagnosis was suggested by Woodruff and Parmley[5] who reported success after surgical excision of the hymen and adjacent minor vestibular glands in 15 cases.[5] In 1985, we saw the term *vulvodynia* defined as the complaint of burning of the vulva without apparent cause.[6] In 1986 Peckham et al.[1] studied tissue blocks from excised surgical specimens and could find no correlation between the site of pain and inflamed minor vestibular glands. They suggested a new name, *focal vulvitis*. Eduard Friedrich[7] thought that term was too general and in February 1987 applied the currently popular term *vulvar vestibulitis syndrome*. This condition certainly is a syndrome rather than a disease because no consistent cause is known and the diagnosis is one made by excluding all known causes. In discussing the possible subsets of vulvodynia or vestibulitis we should also consider vulvar dermatoses, cyclic candidiasis, squamous papillomatosis, and inflammation of Bartholin's ducts.[8, 9] Dr. Goetsch adds three new terms, *borderline*, *primary*, and *secondary* vestibulitis.

I appreciate being reminded of our meager knowledge of this syndrome. If we clinicians continue to combine our experience, we should eventually do better in treating it. As a clinician I offer the following questions for Dr. Goetsch.

1. Because the symptoms described are similar to those of the lactating parturient who is hypoestrogenic and thus finds sexual activity uncomfortably dry, could topical estrogen therapy offer some help?

2. Because the use of antibiotics to treat coliform or streptococcus organisms found in vaginal cultures often make matters worse, it is helpful to use yogurt douches rather than antibiotics to ensure normal vaginal flora?

3. Is there any histologic feature of the mucosa of the posterior fourchette in the crescent between the Bartholin ducts that predisposes the patient to this syndrome?

4. You describe two cases of vestibulitis as a result of fluoroucil therapy as your worst cases. Inasmuch as the cause is known, do these cases properly belong in the report of an entity of unknown cause?

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Dr. Donald H. Minkler, Berkeley, California. When I was a medical student at Harvard in the 1940s, endometriosis was thought to be a relatively rare condition. Then Dr. Joe Meigs at Massachusetts General Hospital began to put safety pins on endometriotic lesions in his surgical specimens so that the pathologists would not overlook them. Dr. Goetsch is similarly telling us that the prevalence of vestibulitis may not be recognized until the appropriate test is applied to all patients. I think her swab test is a useful contribution.

Dr. John A. Enbom, Corvallis, Oregon. One problem with this entity is that we are afraid to ask the question because we do not want the answer. Frequently the patient says somewhat timidly as she passes out the door, "By the way, I have had a problem for a long time but I have been afraid to discuss it." I am certain that these patients are more likely to discuss this with a female gynecologist than with a male.

Dr. Goetsch, in the group you classified as without symptoms, you had some patients who you considered to have positive swab test results. Have you repeated the test on these patients to determine whether this is a repetitive finding or an isolated finding on just that particular day?

Dr. Leroy S. Caspersen, Portland, Oregon. Surgical resection of the vestibular area is a treatment frequently advocated for this problem after the obvious specific causes have been excluded. This treatment adds a surgical scar with its inherent tenderness in a patient already suffering from dyspareunia. Although we are beginning to acknowledge a physical basis for this syndrome, there frequently is a psychologic overlay. I suggest psychologic counseling before undertaking the surgical approach.

Dr. Philip G. Brooks, Los Angeles, California. I have noted recently a tremendous enthusiasm by users of the colposcopic laser to diagnose HPV infections of the vulva and the vestibule. The diagnosis is usually made on the basis of one biopsy showing koilocytosis without any of the other marks of HPV and without either in situ hybridization or viral typing. The diagnosis of HPV has prompted some of these physicians to use surgery, laser, or topical fluoroucil, the last being obviously a very toxic treatment. Dr. Goetsch, in your patients who had concomitant or previous HPV infections, how was the diagnosis made? A diagnosis of HPV infection that is erroneous or merely coincidental should not be part of the vestibulitis syndrome.

Dr. Richard Marchik, Berkeley, California. The trouble is not with the diagnosis of this syndrome, it is with determining the right treatment. I do not think we have any good ones. The symptoms wax and wane almost without regard to what we do. If you wait long enough, most often the symptoms come back. Some people in our speciality have been overtreating this syndrome. In my community some have been very aggressive in the use of the laser to treat this entity by undermining the vaginal mucosa and bringing it down. They talk about all their cures, but I have some of their patients in my practice now who, a few months or years after surgery, have had recurrence of symptoms. By asking a good pathologist to go back and look at the slides, I have found that often the diagnosis of HPV was determined only on the basis of koilocytosis. My good pathologist will tell you that this is not sufficient to make the diagnosis of HPV. With hybridization of deoxyribonucleic acid, HPV cannot be found. The real challenge for us is to find a nonaggressive therapy that works.

Dr. Keith P. Russell, Los Angeles, California. We are all indebted to Dr. Goetsch for bringing the can opener to this picnic. Never have we in this society had so many authorities discussing their views on a subject. We all seem to agree that we are treating a disease but it is very hard to quantitate the symptoms. It seems to me that the diagnosis is made on the basis of the patient's reaction. Dr. Goetsch, in your study was there any attempt to correlate symptoms that morphologic changes such as thickening of Skene's glands or Bartholin's ducts, indicating prior infection?

Dr. Goetsch (Closing). Vulvar pain disorders have seemed a quagmire of complaints with many names and few clarifying characteristics. It is apparent, however, that vulvar vestibulitis does have distinguishing features that set it apart. I prefer to not consider this entity as a subset of vulvodynia because the patients in this study did not have constant pain as do those with vulvodynia. They were without pain except with coital entry or tampon insertion. They did not have chronic itching as do many patients with vulvar condylomata. When burning was the most prominent feature, it was post coital and not continuous with only one exception.

Dr. Broberg, you asked whether topical estrogen might help these patients. Evaluation of treatment was not the goal of this study, but I did look for a relationship of symptoms to hormone status. Neither development of nor worsening of symptoms correlated convincingly with the occurrence of menopause. Both patients with vestibulitis after high-dose fluouroucil treatment were given trials of topical estrogen cream with no improvement. I have a study under way in postpartum patients to determine the proportion in whom dyspareunia develops and which treatments help.

The only role I see for antibiotic treatment of vestibulitis is in the case of a specifically diagnosed problem such as group B streptococcus. Unlike reports of vulvodynia,[1] patients in this study did not relate worsening of symptoms to episodes of antibiotic therapy.

Woodruff and Parmley[2] pointed out that the vestibule is the area of the vulva most likely to react to irritants such as fluouroucil cream. This is also true of the milder irritation of vaginitis. Vaginitis, however, is not the cause of the vestibular pain. I think that these examples support the contention that this area has a greater tendency to become sensitive than does the adjacent vulva or the vagina. With no histologic explanation for the vestibule's heightened level of sensation, even in women without symptoms, I wonder whether it relates to the degree of innervation, something that might vary genetically.

You questioned whether the cases of fluouroucil injury belong in this discussion. I hope that we can increase our understanding of vestibulitis so that it is not considered to have an unknown cause. These two patients had initial chemical burns and tissue destruction, but ultimately they were left with vulvar vestibulitis.

Dr. Enbom asked whether I did repeated swab tests, especially in the group without symptoms. Early in my studies I checked patients a second time if they returned, and my impression was that the examinations varied by perhaps one level of response and by one in the number of positive sites. Because repeating examinations was cumbersome, I chose a cross-sectional design with a single examination for this report. The scale of responses used in this study is more detailed than necessary for clinical use. The women more likely to have symptoms were those with a broad surface area of tenderness, not those with a small spot that was quite painful.

Drs. Casperson and Marchik both express appropriate concern that surgery may be too hastily recommended. Most patients we see in practice have mild to moderate complaints and a large portion have no need for intervention. The application of lidocaine jelly to the vestibule before intercourse may be suggested. Specific causes such as group B streptococcus and obvious lesions of HPV should be treated. In my practice I have suggested surgery to only four patients. One declined, one early patient was treated with laser therapy, and the other two were treated with surgical revision of the fourchette and hymen. I resected only superficially, taking the skin but not resecting subcutaneous tissue or extending the incision down over the perineal body as described by Woodruff and Parmley.[2] This superficial skin revision can be done without scarring, although one must securely fix the edges of the defect during closure. If the incision pulls open and granulation tissue forms, the result may be as painful as the original problem.

I share Dr. Brooks' concern that HPV is overdiagnosed by histology alone. In this study, any patient with a history of treatment for cervical dysplasia, carcinoma in situ, or clinical condylomata were considered to have a presumptive diagnosis of HPV. A few patients did not meet these criteria. DNA hybridization tests were not used. My finding an association between secondary vestibulitis and a history of HPV-related disease points out the need for further investigation with accurate evidence for HPV at the sites of pain. I did note contradictory findings. Colposcopy on five of the nine patients who had both secondary symptoms and a history of HPV showed white epithelium at a site of the swab test in only one patient whose pain had now resolved. Therefore I have not treated any vestibulitis patient for HPV and believe we should be cautious in presuming a causal relationship.

Dr. Russell, I found few morphologic changes in the vestibule. These patients may have erythematous spots or bands of skin, but even these are subtle. The lack of morphologic change raises the question of whether the vestibule has a wider range of sensitivity than we had assumed. We are left with the quandary of whether the tenderness we find in some women represents a pathologic process.

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Gliomatosis peritonei with malignant transformation: A case report and review of the literature

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Gliomatosis peritonei is the implantation of glial tissue within the peritoneal cavity associated with ovarian teratomas. Previous reports have emphasized improved outcomes when these implants are found to be mature, even if the ovarian component is immature. A 16-year-old female with grade 3 immature teratoma was found on two subsequent laparotomies to have extensive peritoneal implantation of mature glial tissue. More than 5 years after the original surgery she was found to have a malignant abdominal glial neoplasm. This case illustrates a rare finding of malignant transformation of previously mature gliomatosis peritonei. (AM J OBSTET GYNECOL 1991;164:1617-21.)

Key words: Gliomatosis peritonei, immature teratoma, malignant transformation

Gliomatosis peritonei, defined as the implantation of mature neural tissue on surfaces of visceral or parietal peritoneum, usually in patients with ovarian teratoma, is a rare condition. Although the mechanism of implantation is unknown, it probably occurs most commonly from extrusion of mature glial tissue through capsular defects in the tumor[1] and is associated with teratomas of all grades. Despite often widespread involvement of peritoneal surfaces, gliomatosis peritonei is reported to impart an improved prognosis even in immature teratomas.[1, 2] A unique case is presented with mature (grade 0) glial implants documented both 10 and 22 months after the original diagnosis of grade 3 immature teratoma was made. Malignant transformation of these implants developed later. Previous reports of this phenomenon are reviewed.

Case report

A 16-year-old nulliparous female was admitted with abdominal pain and a large pelvic mass. At laparotomy there was a 20 cm, twisted left ovarian tumor with a 7 cm rupture in the capsule. A left salpingo-oophorectomy was performed. There were no obvious peritoneal implants noted; however, a staging laparotomy was not performed. The α-fetoprotein value was elevated at 557 ng/ml, while the β-subunit of human chorionic gonadotropin and chemistry values and chest x-ray film were normal. The tumor was reported as grade 3 immature teratoma.

The patient was treated with four cycles of cisplatin, vinblastine sulfate, and bleomycin and remained asymptomatic until 10 months after the original operation. At that time she was readmitted with acute, left lower quadrant pain. She had a large, tender cul-de-sac mass. Intravenous pyelogram, chest x-ray, and hematologic and chemistry values were normal. Test results for β-subunit of human chorionic gonadotropin and α-fetoprotein were negative. Laparotomy revealed a 10 × 6 × 5 cm cul-de-sac mass that was multilocular without excrescences. There were multiple peritoneal nodules from 2 mm to 2 cm in size over the bladder peritoneum, cul-de-sac peritoneum, posterior broad ligaments, uterosacral ligaments, and right fallopian tube. She underwent excision of the mass, pelvic and paraaortic lymphadenectomy, omentectomy, appendectomy, multiple peritoneal biopsies, including the diaphragms, for staging, and excision of multiple peritoneal nodules. The pathology report was of a mature teratoma with mature glial implants. No further therapy was given.

She was readmitted 22 months after the original laparotomy for removal of a grade 3 immature teratoma and 13 months after the second operation for removal of mature teratomatous tissue, with a new, right-sided 4 cm pelvic mass. Exploratory laparotomy showed a 4 × 5 cm, thick-walled, right ovarian cyst without excrescences. There were numerous small nodules <5 mm confined to the peritoneum of the small bowel, bladder, and rectosigmoid colon, but the upper abdomen was normal. The right ovarian cyst was removed and multiple biopsies of the pelvic nodules were done. The pathology report revealed a corpus luteum cyst and mature glial implants.

She underwent follow-up without additional therapy until 5½ years after the original operation, when she was readmitted because of increasing enlargement of a pelvic mass thought to be recurrent mature glial implants. The tumor markers β subunit of human chorionic gonadotropin, α-fetoprotein, and CA 125 all were normal. At operation there was acites, as well as

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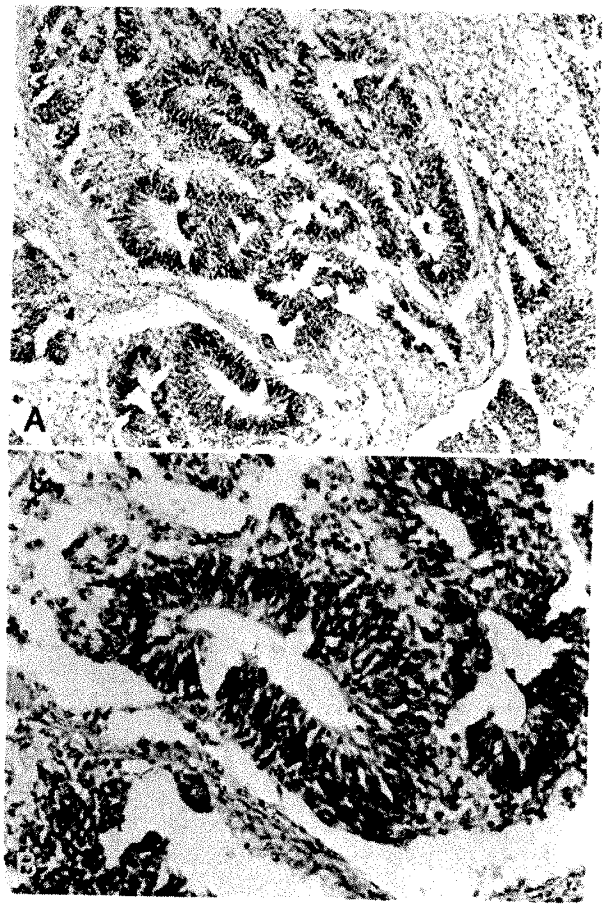

Fig. 1. First laparotomy. **A,** Grade 3 immature ovarian teratoma with numerous neuroepithelial rosettes. **B,** Neuroepithelial rosettes. (Hematoxylin and eosin. Original magnification ×200.)

widespread metastatic seeding of the anterior abdominal peritoneum and the surfaces of the large and small bowel. The mass was a malignant tumor with glial differentiation infiltrating the abdominal wall muscuature.

The patient was treated with cisplatin, etoposide, and bleomycin for a total of four courses and later switched to VAC (vincristine, Actinomycin D, and Cytoxan). She showed no response to VAC and was later treated with palliative radiation to the anterior abdominal wall mass. She received further chemotherapy with BiCNU and 6-thioguanine. All therapy failed and she died of disease 7 years after the original diagnosis.

Pathologic findings. The original left ovarian tumor measured 17 × 15 × 9 cm and weighed 1020 gm. The tumor was solid and cystic, and the capsule was ruptured. Light microscopic sections showed a grade 3 immature teratoma with abundant immature mesenchymal elements, cartilage, and neuroepithelium (Fig. 1, *A* and *B*).

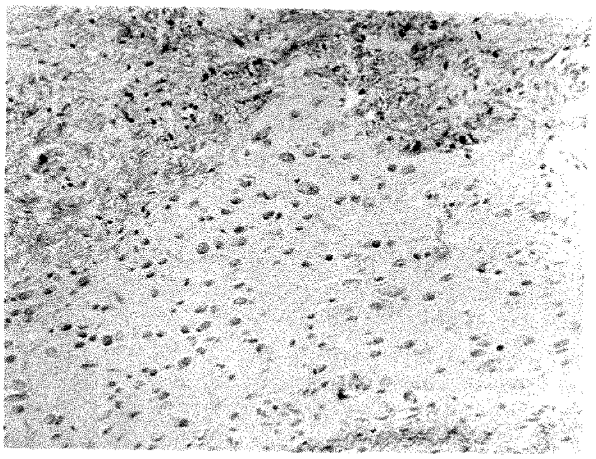

Fig. 2. Second laparotomy. Mature, grade 0 glial nodule, with scattered neurons. (Hematoxylin and eosin. Original magnification ×200.)

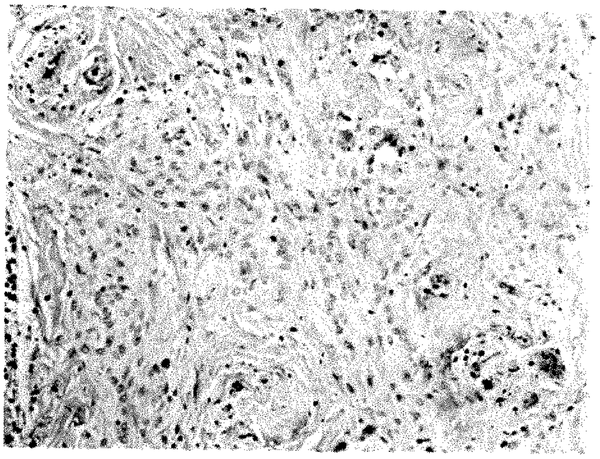

Fig. 3. Third laparotomy. Mature grade 0 glial implant. (Hematoxylin and eosin. Original magnification ×200.)

At the second operation 10 months later, multiple tissue fragments from the cul-de-sac measuring up to 10 cm in diameter were received. Extensive sectioning showed a fibrous, collagenous background, with numerous mature glial-neural nodules without evidence of immature elements (Fig. 2). The tissue contained some small cystic structures lined by cytologically bland, mature, ciliated and nonciliated columnar cells. The combination of mature glia and benign epithelial cysts was interpreted as mature teratoma. Multiple biopsies of smaller nodules from the surface of the cecum, descending colon, and bladder showed identical mature glial tissue, without immaturity. Specimens from the omentum, diaphragms, and pelvic lymph nodes were negative for malignant tumor.

The third laparotomy, performed 22 months after the first operation, revealed a corpus luteum cyst of the right ovary. Multiple biopsy specimens from the surface of the rectosigmoid colon, bladder, and small bowel showed mature glial implants, without immaturity or other teratomatous elements (Fig. 3).

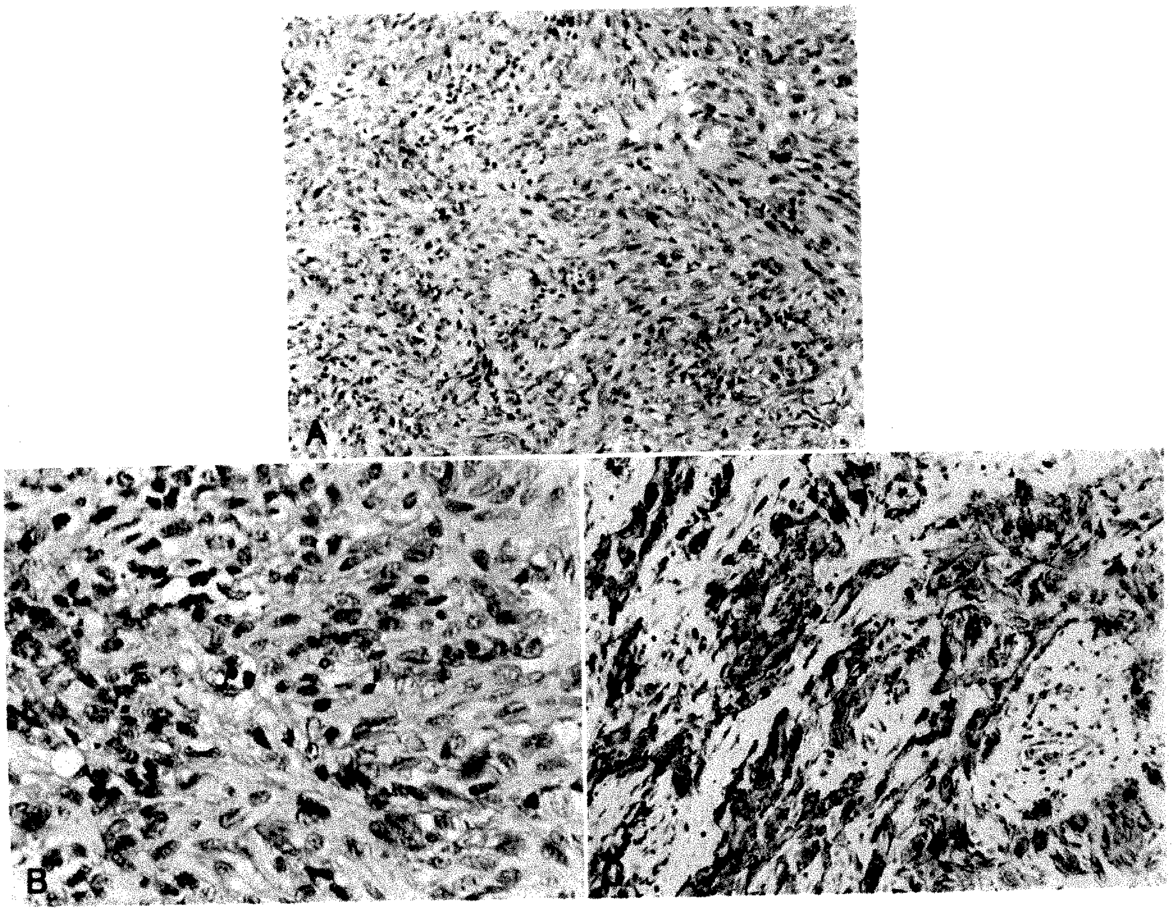

Fig. 4. Fourth laparotomy. **A,** Cellular, high-grade malignant glial tumor. (Hematoxylin and eosin. Original magnification ×200.) Compare with Figs. 2 and 3. **B,** Malignant glial tumor. (Hematoxylin and eosin. Original magnification ×510.) **C,** Tumor cells positive for glial fibrillary acid protein. (Original magnification ×200.)

The fourth laparotomy, performed 67 months after the original operation, showed a large anterior abdominal wall mass, as well as multiple nodules on the large and small bowel and in the area of the previous omentectomy site. The microscopic sections of these nodules revealed a highly cellular malignant tumor, with round and spindle-shaped cells exhibiting marked nuclear pleomorphism that infiltrated the anterior abdominal wall skeletal muscle (Fig. 4, *A* and *B*). The tumor did not form malignant mesenchymal structures or definite teratomatous foci, and there were no neuroepithelial rosettes, as seen in the original immature ovarian teratoma. The tumor cells were cytokeratin negative and were positive for both S-100 protein and glial fibrillary acid protein (Fig. 4, *C*). We interpreted this tumor as a malignant glial neoplasm, more analogous to a malignant glioma than to metastatic immature teratoma.

Review of literature

Gliomatosis peritonei is the implantation of mature glial tissue on the peritoneal surfaces, usually in association with solid ovarian teratomas. Proskauer[3] reported in 1946 the first series of 10 cases of this rare condition. In 1982 Truong et al.[4] reported two new cases and reviewed the world's literature, which at that time consisted of 30 cases. Since that time, several more cases have been reported, including a recent series of 13 patients reported by Harms et al.[5]

The prognosis of immature ovarian teratoma is closely associated with tumor grade.[6] The primary tumor and implants are graded according to the criteria of Thurlbeck and Scully,[7] which was later modified by Norris et al.[6] In the series of Norris et al. of 58 patients, 5-year survival rates for patients with grades 1, 2, and 3 were 82%, 63%, and 30%, respectively. However, in contrast to these survival figures, patients who have immature ovarian teratomas in association with mature glial implants appear to have a much improved prognosis.[1, 5] This latter statement is clearly dependent on two important factors: (1) Peritoneal surfaces and omentum must be thoroughly sampled histologically, including diaphragmatic surfaces; (2) all of the implants must be mature, i.e., without immature elements.

If these two conditions are met, the prognosis of the disease is excellent.

There are three cases in the literature that reportedly had adverse outcomes. Albites[8] reported a case in 1974 of a 9-year-old girl with a 19 cm ovarian teratoma described as mature, with grade 0 peritoneal glial implants. The report does not describe extensive peritoneal sampling. The patient was readmitted 14 weeks later with a large, left upper quadrant mass. The patient died 5 months after the initial operation. The rapid recurrence of disease and death of the patient suggest that areas of immature teratoma on the peritoneal surface were not sampled. Khoo et al.[9] in 1978 described a 17-year-old girl who presented with a 15 cm grade 2 immature ovarian teratoma, with grade 2 peritoneal glial implants; she died of disease 12 months after presentation. This case again fails to meet the two previously listed criteria because the glial implants were not completely mature. Nielsen et al.[10] reported four cases of gliomatosis peritonei in 1985. Their case 3 was a 29-year-old woman with a 25 cm, grade 3 immature teratoma, with grade 0 peritoneal implants of the omentum. However, diaphragmatic and peritoneal implants were present but not extensively sampled. One month later the patient underwent a second operation, and a 15 cm malignant teratoma was found. We interpret this as recurrent immature teratoma because of the rapid recurrence and inadequate sampling, rather than malignant transformation of the grade 0 glial implants.

Comment

The patient described in this article represents a unique case. After initial presentation with a grade 3 immature ovarian teratoma (the patient did not initially undergo staging), two subsequent laparotomies revealed mature peritoneal glial implants without areas of immature teratoma. In addition to extensive lymph node dissection and omentectomy, extensive peritoneal tissue sampling was performed during each of these laparotomies. A final laparotomy, performed >5 years after initial presentation, revealed a cytologically malignant glial proliferation. The glial nature of these malignant cells was confirmed by the expression of glial fibrillary acid protein. We interpret this case as an example of malignant transformation of benign peritoneal glial implants, which resulted in the eventual death of the patient.

In summary, gliomatosis peritonei is a rare condition, that generally imparts a favorable prognosis to patients with immature ovarian teratomas. Therapy should be directed by the grade and stage of the primary tumor and not by the glial implants, if they are extensively sampled and all are mature.[1, 4] If patients undergo extensive staging, peritoneal implants are well sampled, and histologic description shows the implants to be completely mature, a benign clinical course is to be expected. The current case appears to be a unique exception to the excellent prognosis of this condition and emphasizes the need for continued clinical evaluation and long-term follow-up, even in the face of mature peritoneal glial implants.

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Discussion

DR. WILLIAM CRISP, Phoenix, Arizona. Gliomatosis peritonei is defined as metastatic implantation of neural glial tissue on peritoneal surfaces, usually in patients who have an immature ovarian teratoma. This condition is rarely found with mature teratomas, despite the fact that neural tissue may be found as a component in >30% of mature teratomas. Grossly, gliomatosis peritonei has the appearance of stage 3 ovarian carcinoma and is associated with an adherent capsule or a capsular defect in the parent teratoma in >90% of cases. It is important therefore to excise all ovarian teratomas intact. The potential occurrence of gliomatosis peritonei raises the question of whether the increasing incidence of laparoscopic ovarian cystectomy is desirable.

After an adequate sampling of the metastatic glial implants is taken for pathologic examination, all the implants should be removed. I think that this is best accomplished by using a Cavitron ultrasonic surgical aspirator (Cooper Biomedical Corp.). If the peritoneal implants are histologically mature, they are considered benign, provided that an adequate sampling of the ectopic neural tissues has been done to exclude the presence of germ cell elements and other types of neural tissue. Robboy and Scully[1] have published a classification with a scale from mature to immature that relates to the prognosis. The behavior of ectopic neural tissue as it ages is difficult to predict, as evidenced by the case under discussion.

Adjuvant therapy of malignant gliomatosis peritonei[2] with irradiation and chemotherapeutic regimens has been ineffective. However, there have been promising reports in the neurosurgical literature of immunologic therapy of ectopic malignant glial tissue.[3] Adoptive transfer of autogenous lymphokine-activated killer cells induced in vitro with recombinant interleukin-2 has been used in three patients with central nervous system malignant meningeal gliomatosis. This treatment was effective in reducing all central nervous system symptoms and in eliminating malignant cells from the cerebrospinal fluid. Follow-up on these patients has been for 3 years, and ectopic neural tissue is known to be a continuing threat that demands vigilant follow-up.

Dr. Shefren, what amount of ectopic glial tissue was left at the primary and other operations? Was immunotherapy considered?

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Dr. Shefren (Closing). Immunotherapy was not considered in this patient, but it does seem to be something that might be considered for what has otherwise been unsuccessful treatment of this disease.

The use of potassium-titanyl-phosphate laser for laparoscopic removal of ovarian endometrioma

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Los Angeles, California

From January 1989 through January 1990, 31 patients thought to have ovarian endometrioma at ultrasonography were treated by operative laparoscopy and potassium-titanyl-phosphate laser. The endometriomas ranged from 2 to 9.2 cm in diameter by preoperative transvaginal ultrasonography. The operative procedure used was a three-puncture technique with a 600 μm fiber attached to a potassium-titanyl-phosphate laser. The power setting was 10 W with a continuous pulse. In all cases endometriomas were opened, drained, and irrigated, and the potassium-titanyl-phosphate laser was used to vaporize or remove the entire cyst lining. The ovarian edges were inverted with the laser at the end of the procedure. With one exception, there was no recurrence of endometrioma at 1, 3, and 6 months after the procedure, on the basis of transvaginal ultrasonography or laparoscopy. In one case, 4 months after the initial laparoscopic procedure, exploratory laparotomy was performed for resection of a large, recurrent endometrioma. The use of the potassium-titanyl-phosphate laser allows the laparoscopic surgeon an aggressive approach to ovarian endometriomas that in the past necessitated exploratory laparotomy. (AM J OBSTET GYNECOL 1991;164:1622-8.)

Key words: Potassium-titanyl-phosphate laser, endometrioma

Pelvic endometriosis is a commonly identified factor during the evaluation of the infertile woman. Controversy still remains as to therapeutic approach and outcome concerning the infertile state.[1-4] Normally endometriosis can be diagnosed only at the time of pelvic visualization by laparoscopy, culdoscopy, or exploratory laparotomy. However, the presence of ovarian involvement may be suspected on a clinical basis by visualization of cystic structures with transvaginal ovarian ultrasonographic scanning.[5]

With the advent of endoscopic laser technology, an aggressive, less invasive approach to pelvic endometriosis has become available. In the past the presence of ovarian endometrioma usually necessitated exploratory laparotomy and ovarian cystectomy or oophorectomy.[6] This report explores the use of an aggressive laparoscopic approach to ovarian endometriosis with laser ablation or resection.

Material and methods

Patient group. The study group was composed of 31 infertile women who were referred to our center from January 1989 through January 1990 for ovulation induction, gamete intrafallopian transfer, or in vitro fertilization and embryo transfer. The duration of infertility in these patients ranged from 18 to 68 months. The age range was 26 to 43 years, with a mean of 35.7 years. Before ovarian stimulation, all patients underwent transvaginal ovarian ultrasonographic scanning of the ovaries with ATL Ultramark 4 equipment and a 5 MHz transvaginal ultrasonographic probe (ATL, Bothell, Wash.). Visualized cystic-solid ovarian masses ranged from 2 to 9.2 cm in diameter. A presumptive diagnosis of ovarian endometrioma was made on the basis of ultrasonographic appearance (Fig. 1).

All patients were placed on a regimen of leuprolide acetate (Lupron, TAP, Chicago) for 6 weeks to 3 months before the laparoscopic procedure. All patients had complete infertility evaluations before initiation of medical suppression, including full male factor evaluation. Before surgical intervention, patients signed informed consents for laparoscopy, possible exploratory laparotomy, and possible oophorectomy.

Operative instruments. A potassium-titanyl-phosphate laser (KTP/532, Laserscope, Santa Clara) was used in all cases. The laser energy was delivered through a 600 μm core flexible quartz fiber. The usual energy level was 10 W with a continuous pulse mode. The fiber was passed through the operating channel of a 10 mm Wolf operating laparoscope (Richard Wolf Medical Instruments Corp., Rosemont, Ill.). Secondary instruments (5 mm) were placed in the pubic hairline in the right and left lateral margins under direct visualization, after transillumination of the potential puncture site. Grasping and suction/irrigation instruments were used through these 5 mm ports.

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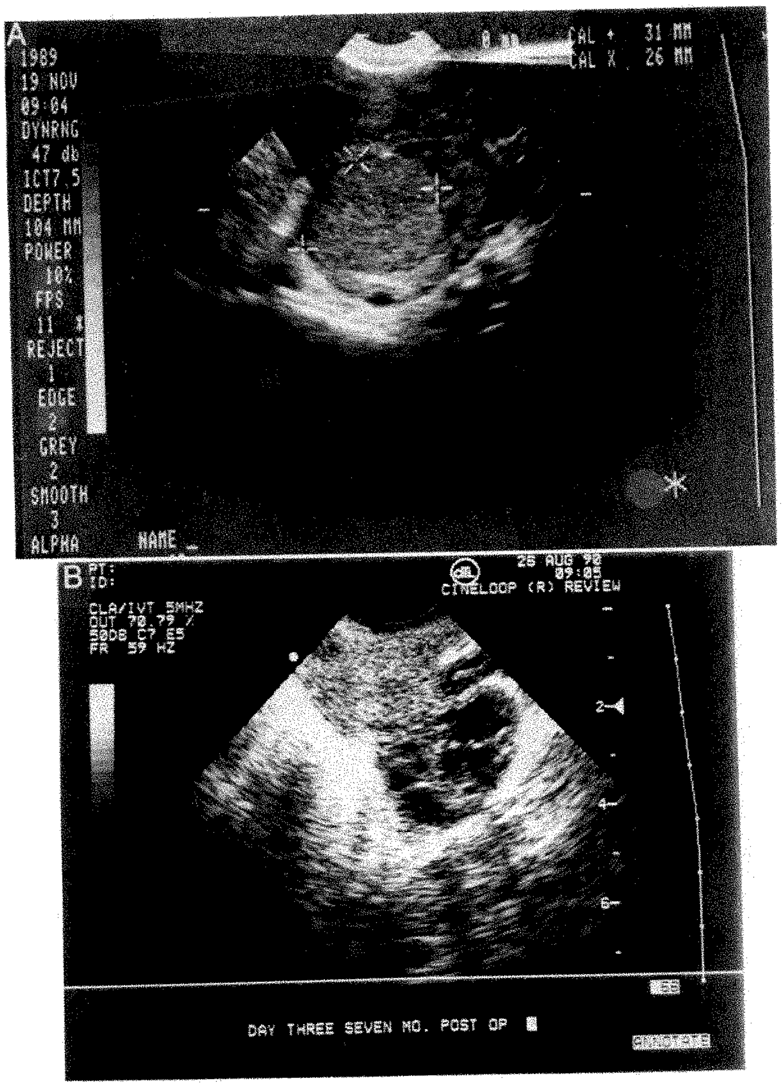

Fig. 1. A, Preoperative ultrasonographic scan of 3.1 × 2.6 cm left ovarian endometrioma. **B,** Transvaginal ultrasonographic scan of same patient. Normal left ovary on day 3 of cycle, 7 months after laparoscopic laser resection.

Operative procedure. Salpingolysis and ovariolysis were performed in all but two cases. These cases had a single endometrioma with no adhesions, ovarian fixation, or peritoneal implants of endometriosis. In the remaining 29 operative procedures, ovarian fixation was present. Before the cyst was opened, the structure was aspirated to ensure the diagnosis. Ovariolysis was then performed with the potassium-titanyl-phosphate laser to completely mobilize the ovary and endometrioma. The endometrioma was opened in a linear fashion over the area of marked protrusion from the ovarian surface, and the cyst was thoroughly irrigated and inspected for areas of excrescence. With operative scissors, a portion of the cyst wall was removed for pathologic evaluation (Fig. 2, *A*).

In cases where the endometrioma was ≤ 4 cm in diameter, the cyst wall was opened and the potassium-titanyl-phosphate laser was used to photocoagulate or vaporize the entire cyst lining. In larger endometriomas the cyst wall was dissected from the ovarian stroma and removed intact. Unipolar coagulation was used for hemostasis. Once the endometrioma was removed, the ovarian capsule was retracted by directing the laser to the undersurface of the cut edge of the ovary and effectively inverting the ovarian edge, thus decreasing the exposure of denuded ovarian surface (Fig. 2, *B*).

Results

Laparoscopy was performed after a presumptive diagnosis of ovarian endometrioma, on the basis of transvaginal ultrasonographic visualization in all 31 cases. Thirty of these patients had endometrioma and stage

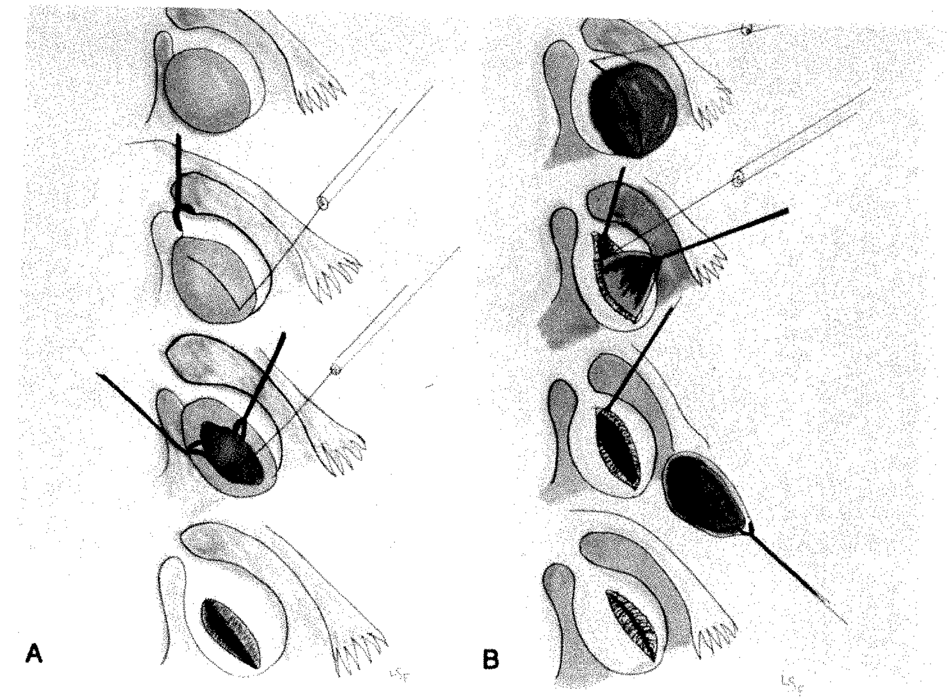

Fig. 2. A, Laparoscopic technique for laser ablation of endometrioma ≤4 cm in diameter. **B,** Laparoscopic laser cystectomy for endometrioma.

III or IV endometriosis (revised American Fertility Society classification); one patient had a 2 cm cystic teratoma (dermoid). All lesions (including the dermoid) were removed by operative laparoscopy and potassium-titanyl-phosphate laser. All procedures were performed by the author with the patient under general anesthesia in an outpatient surgical facility. Patients were discharged the day of operation, after an average recovery time of 4.3 hours. The surgical time ranged from 40 to 103 minutes, with an average of 52 minutes per case. The blood loss did not exceed 100 ml in any case and averaged 30 ml. All cases were completed laparoscopically, and there were no complications such as bowel, bladder, or vascular injury.

Time of follow-up ranged from 6 to 21 months. All 31 individuals have had follow-up transvaginal ultrasonographic examinations at 1, 3, and 6 months (Fig. 1, *B*). A recurrent endometrioma was observed in only one case; it was removed by exploratory laparotomy and ovarian cystectomy (Table I).

Twenty-three of the 31 patients have actively attempted pregnancy after the laser surgical procedure. Among these, seven pregnancies have occurred. In three patients, subsequent laparoscopy has been performed for gamete intrafallopian transfer, and no adhesions or evidence of endometrioma recurrence was visible.

Comment

This report demonstrates the usefulness of a laparoscopic approach for treatment of ovarian endometriomas. Moreover, it suggests that identification of ovarian endometriomas is enhanced with transvaginal ultrasonographic visualization of the ovaries in an infertile population.

The advantage of a laparoscopic approach to ovarian endometriomas is twofold. First, the patient avoids the physical and financial expense of hospitalization and exploratory abdominal surgery. Second, the risk of adhesion formation, theoretically and practically, should be decreased with a laparoscopic approach (Marrs RP. Unpublished data). In this study a subsequent laparotomy for endometrioma resection was required in only one of the cases. This patient (case 18) had a history of two previous laparotomies for treatment of stage IV endometriosis. At the time of laparoscopic resection, complete mobilization and access to the one remaining ovary could not be accomplished. In retrospect, a laparotomy approach would have been more suitable. However, in the remaining cases, where complete access and mobilization of the ovaries and cul-de-sac were accomplished, no recurrences have been seen. This compares favorably with the earlier report of Reich and McGlynn[7] using a laparoscopic approach.

Table I. Surgical outcome

Patient No.	*Age (yr)*	*Stage of endometriosis*	*Mass size by ultrasonography (cm)*	*Time from operation (mo)*	*Ultrasonographic follow-up*	*Pregnancy*
1	36	III	3.2	21	Clear	Delivered
2	37	III	2.8	20	Clear	Not attempting
3	40	IV	5 3	16	Clear	No
4	30	III	5	14	Clear	No
5	39	IV	3 2.5	14	Clear	No
6	40	III	2.7	15	Clear	Not attempting
7	43	III	2	14	Clear	No
8	35	III	3.5	14	Clear	No
9	39	IV	3.8	13	Clear	Spontaneous abortion Delivered
10	38	IV	2 4	14	Clear	Currently pregnant
11	36	III	5	13	Clear	No
12	35	III	2	12	Clear	Not attempting
13	32	III	3	11	Clear	Spontaneous abortion
14	43	III	3.6	10	Clear	No
15	33	III	4	8	Clear	No
16	31	III	4	7	Clear	No
17	37	III	2.5	9	Clear	No
18	37	IV	3.5 3.1 2.6	8	Recurrence	No
19	34	III	2 1.5	9	Clear	No
20	29	III	2	8	Clear	No
21	35	III	3 2	8	Clear	Currently pregnant
22	26	III	4	8	Clear	Not attempting
23	39	No endometriosis; dermoid	2	8	Clear	No
24	32	IV	7	9	Clear	Currently pregnant
25	33	IV	9.2 3.1 2.6	10	Clear	No
26	38	III	2.2	9	Clear	Not attempting
27	37	III	3	6	Clear	Not attempting
28	34	III	4	6	Clear	Not attempting
29	38	III	3	6	Clear	No
30	38	III	4	6	Clear	Not attempting
31	34	III	4	6	Clear	No

The surgical approach settled on was initially somewhat arbitrary but in retrospect has been reliable. Endometriomas <4 cm are easily ablated with the fiber-delivered potassium-titanyl-phosphate laser. In lesions of this size, when an attempt at removing the cyst wall was made, bleeding from the adjacent ovarian tissue was usually encountered. Therefore the endometrioma was opened, drained, and irrigated, and the potassium-titanyl-phosphate fiber was used in a "paintbrush" fashion to completely photocoagulate all visible surfaces of the endometrioma lining. Although this technique can be used for endometriomas of any size, the time factor is more prohibitive in the larger lesions. It was easier to separate and remove the cyst wall in endometriomas >4 cm in diameter. In the case of the individual 9.3 cm endometrioma, the cyst wall was easily removed, and the remaining normal ovarian tissue was trimmed and left in place.

The pregnancy rate thus far in this small series of patients with a short follow-up is encouraging. Of the 23 patients attempting to conceive, seven patients have succeeded, with five either currently pregnant or already delivered. Even though only three patients have had laparoscopy after endometrioma resection, only minimal disease was visualized. This, combined with the number of patients conceiving, again suggests that a laparoscopic laser approach for this type of pelvic disease offers a very successful treatment modality.

These results compare favorably with a report by Kojima et al.[8] in which a neodymium:yttrium-aluminum-garnet laser was used for laparoscopic treatment of ovarian endometrioma.

In summary, a laparoscopic laser approach to ovarian endometrioma can be performed safely and efficiently. Surgeons experienced in operative laparoscopy and laser laparoscopy can use this approach without difficulty. The advantage to the patient is overwhelming, and patient acceptance is high. Thus the necessity for multiple laparotomies in patients with ovarian endometrioma is now avoidable.

Addendum

Since the preparation of this manuscript, three more patients have ongoing pregnancies and seven other patients have had second-look laparoscopy demonstrating no adhesions.

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Discussion

Dr. Simon Henderson, San Francisco, California. Dr. Marrs has very elegantly presented us with another facet of advanced operative laparoscopy, namely, the destruction and removal of ovarian endometriomas with the potassium-titanyl-phosphate laser in women pretreated with leuprolide acetate. He has told us that seven pregnancies occurred among the 23 (30%) patients and that one of 31 (3%) had a large, recurrent endometrioma 4 months after the procedure, necessitating laparotomy and ovarian cystectomy. It will be interesting to hear from Dr. Marrs in another 2 to 3 years whether any of his other patients required further surgical treatment, since Hammond et al.[1] reported in 1976 that laparotomy and conservative surgery in women with severe endometriosis resulted in a 100% surgical recurrence rate, with the average time from the initial surgery to recurrence being 40 months. The original gold standard for pregnancy rates was set by Buttram[2] (1979) who performed laparotomy, ovarian cystectomy, and removal of as much endometriosis as possible in 47 patients with severe endometriosis. He achieved a 40% pregnancy rate with 15 months of follow-up.

Laparoscopic treatment of ovarian endometriosis has undergone a metamorphosis since Frangenheim[3] reported in 1978 that large endometriomas could be punctured and the contents aspirated, but 40% of these patients required subsequent surgical removal of endometrioma via laparotomy. Mettler and Semm[4] in 1983 first described laparoscopic ovarian cystectomy for endometriomas, involving removal of the capsule with scissors and closing the ovary with sutures. Reich and McGlynn[5] in 1986 reported laparoscopic excision of endometriomas using the unipolar knife electrode and leaving the ovary unsutured. Their patients were not pretreated with danazol or other drugs, and 10 of 21 (48%) achieved term pregnancies, with up to 8 years of follow-up. However, seven of 21 (33%) patients required further surgery for endometriosis. Nezhat et al.[6] in 1988 highlighted the need for excision of the cyst wall, since their recurrence rate was 50% in a group of patients treated with aspiration and irrigation of the endometrioma, compared with 8% for the group who had the cyst wall excised with carbon dioxide laser under video control. The pregnancy rates were 62.5% and 80%, respectively, for the two groups, both of which were treated postoperatively with danazol for 30 days. In June 1990 Kojima et al.[7] reported on 16 patients with infertility who had endometriomas removed with neodymium:yttrium-aluminum-garnet video laser laparoscopy without preoperative or postoperative treatment with danazol or gonadotropin-releasing hormone agonist. They used a cutting probe for opening the endometrioma and a lysing probe to dissect the cyst wall. Six (37.5%) patients achieved pregnancy and one (3%) patient underwent a subsequent laparotomy for removal of recurrent endometrioma. Four of nine patients who had second laparoscopies had mild periovarian adhesions. In both of these last-mentioned reports the ovary was left open.

On reviewing all my 113 cases of operative video laparoscopy between June 1989 and May 1990, I found 20 cases of ovarian endometriomas, 19 of which were treated with carbon dioxide laser vaporization, excision without closing the ovary, or pretreatment or posttreatment administration of gonadotropin-releasing hormone agonist or danazol. The large smoke production was not a problem with carbon dioxide insufflation flow rates of up to 6 L/min and use of the suction irrigation probe. The one patient not so treated had severe pelvic endometriosis with bilateral endometriomas 5 to 6 cm in diameter, which were completely adherent to the bowel and sidewall. These cysts were drained laparoscopically, and the patient was treated with 3 months of danazol before ovarian cystectomy and tubal microsurgery. In one of the 19 patients who had initially been treated laparoscopically for bilateral endometriomas of 5 cm each, a unilateral endometrioma subsequently redeveloped; she required danazol treatment for 2 months and subsequent laparotomy and ovarian cystectomy. Five of the 14 patients (36%) with ≥6 months of follow-up are pregnant.

I should also mention that Dr. Marrs' average operating time of 52 minutes was considerably lower than the operative times reported by Reich and McGlynn[5] and Kojima et al.[7], 80 and 90 minutes, respectively, and my own of 90 minutes.

The choice of laser is very individual, and Dr. Marrs has given his reasons for using the potassium-titanyl-phosphate laser. My reason for using the carbon dioxide laser is that it passes through the operating laparoscope almost along one's line of vision, often reducing the need for additional abdominal punctures and the need for another hand by one. The carbon dioxide laser has unsurpassed cutting and dissecting abilities; there is no chance of a fiber or probe breaking off. If you can see the structure to be treated, you can lase it, and if greater hemostasis is required, a needle electrode with pure coagulation or a blend of cutting and coagulation is always available through a secondary puncture.

In summary, Dr. Marrs' results compare very favorably with the results quoted by a variety of authors using electrosurgery or several different types of laser to remove endometriomas laparoscopically. I would not be surprised to see that his pregnancy and endometrioma recurrence rates are higher after longer follow-up.

My questions for Dr. Marrs are: (1) Did you use a video camera? (2) How many of your patients who conceived did so with the help of clomiphene, Pergonal, in vitro fertilization, gamete intrafallopian transfer, or other procedures? (3) Is it possible that vaginal ultrasonography will miss significant intraovarian endometriosis that might be capable of creating new endometriomas in the future?

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Dr. James C. Caillouette, Pasadena, California. This procedure has many of the characteristics of an ovarian wedge biopsy. As you all know, the ovarian wedge biopsy for treatment of polycystic ovaries was used with great success for many years, until it lost favor because of the problem of adhesion formation. I am impressed, Dr. Marrs, that your procedures resulted in few adhesions, and therefore I have two questions: (1) Is there a place for the new product Interceed (Johnson & Johnson, Newark, N.J.) to lessen the likelihood of adhesions in this type of procedure? (2) Should we once again consider the possibility of ovarian wedge resection for the treatment of polycystic ovaries in those cases where there is no response to medical therapy?

Dr. Jack R. Lamey, Seattle, Washington. Medical therapy only, with danazol or gonadotropin-releasing hormone analogs, is an option for treatment of endometriomas measuring <4 cm. Dr. Marrs, what is the relative efficacy of medical therapy and laser surgery with regard to recurrence rates and pregnancy rates?

Dr. Philip G. Brooks, Los Angeles, California. Several studies[1, 2] have demonstrated hidden endometriosis, that is, microscopic endometriosis seen in random biopsy specimens of areas of peritoneum without visible lesions in patients who do have visible implants elsewhere. Dr. Marrs, would not medical treatment in conjunction with laparoscopic treatment improve pregnancy rates, because of the presence of endometriosis that is not treated with laser because it cannot be seen?

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Dr. Louis A. Vontver, Seattle, Washington. Dr. Marrs, please comment on two questions. In one of the 40 cases in your series, the operative diagnosis was a dermoid, although the ultrasonographic examination was consistent with endometriosis. As the average age of your patients increases, the risk of an ovarian malignancy also increases. What happens if you open an ovarian lesion and find a carcinoma? (2) Should we have concern regarding possible carcinogens created by carbon particles left in an ovary, the capsule of which is also left open? Are you aware of any data on whether this procedure causes an increased risk for malignant transformation at a later date?

Dr. Marrs (Closing). Dr. Henderson, because of the nature of endometriosis, we are going to see recurrences among patients who do not conceive, and these will be at a rate that is unrelated to the treatment modality. Whether you do laparotomy with ovarian cystectomy, laparoscopic resection, or medical suppression, you are going to find recurrences at a later date. Even among those who do conceive, we have seen recurrences at a later date. The literature does not suggest that there is going to be a higher recurrence rate with laparoscopy.

The potassium-titanyl-phosphate laser has some advantages over the carbon dioxide laser. It is a more penetrating laser, and therefore you have more complete ablation of the cyst wall if you do not remove it. It has coagulating and vaporizing ability, and thus there is less need for electrosurgical coagulation.

Since all of these patients had stage III or IV endometriosis by the American Fertility Society revised classification, the largest amount of time in these procedures is spent in mobilizing the ovaries. Once this has been done, the ablation or resection of the endometrioma is relatively quick. We work hard to reduce the operating time because of our operating room schedule. First of all, the instruments and solutions are prepared and set out preoperatively so that the staff does not have to run to different rooms looking for them. Second, I do not work from the video screen but look through the laparoscope and use a beam-split video camera so that the assistant can view the procedure.

Dr. Caillouette, I have used Interceed in a number of other patients who had extensive laparoscopic lysis of pelvic adhesions or a laparotomy for myomectomy or aggressive adhesion lysis. It is easy to use and, if you can place it in over the ovarian lesion, it may further reduce the formation of adhesions.

I have not done a large number of second-look laparoscopies to date, but in those I have done I was surprised to see only a few, very filmy adhesions around the ovaries or anywhere else for that matter. I would not propose resurrecting wedge resection. There are very limited indications for surgical treatment of polycystic ovary syndrome and I would exhaust all other possibilities before using it. There are also some data in the literature[1] that laparoscopic laser drilling of the ovary in multiple sites offers some benefit to those patients with polycystic ovary syndrome who fail to respond adequately to ovulation-induction drugs.

Dr. Vontver, the risk of malignancy is always a concern. We examine these patients by ultrasonography and make a presumptive diagnosis at the time of laparoscopy. In this series no cases underwent laparotomy because of the appearance of the lesion. Even the dermoid was apparent when we aspirated the fluid contents. However, there will be patients with malignancy, and the question is whether you create problems by identifying the lesion at laparoscopy and then doing a laparotomy for treatment. I do not know. We are very cautious about doing these procedures in the older age group.

I do not know whether carbon particles left after laser may be potential teratogens in ovarian tissue. To be on the safe side, we do copious irrigation after any laser procedure. With the ovary, we irrigate with 3 to 5 L of lactated Ringer's solution.

Dr. Lamey suggested the use of medical suppression only for endometriomas ≤4 cm. We have done follow-up examinations of patients, who were found to have endometriomas at the time of later surgery, for up to 3 months while they received suppressive therapy and have seen absolutely no change in size or ultrasonographic consistency of the lesion in the ovary. I know of no data to support the contention that medical treatment will remove endometriomas in the ovary. It is a good preoperative treatment, but surgical removal is the only known therapy for elimination of endometriomas.

Dr. Brooks, I concur that systemic treatment before or after laparoscopy should enhance the pregnancy outcome, because it treats the microscopic foci of endometriosis that we know exist and cannot treat because we cannot see them. The existence of these microscopic lesions is one reason we have used combination therapy in cases with extensive pelvic endometriosis. An advantage of preoperative medical therapy is that the pelvic cavity is less vascularized and less edematous. I also think there is a reduction of postoperative adhesions. A disadvantage of preoperative treatment is that you may suppress endometriosis to the extent of making some lesions invisible and therefore they are not treated by laser at the time of the procedure.

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Organ transplantation and pregnancy

A case report and review

E. Paul Kirk, MD
Portland, Oregon

The 1980s has been described as the "decade of transplantation" and organ transplantation as a "paradigm of medical progress." The considerable improvements in immunosuppression that have been made in the last few years have led to an increased repertoire of organ-to-organ combination transplants, more procedures, and markedly increased survival time for both grafts and recipients. Inevitably, there will be an increased number of women recipients who later undergo pregnancy. Although there is a wide experience with pregnancy after renal transplantation, there are as yet relatively few reports of pregnancy after other major organ transplants. A case is presented of a 22-year-old primiparous woman who elected to conceive and complete a pregnancy 2 years after cardiac transplantation because of cardiomyopathy. The pregnancy was complicated by a rejection episode early in the third trimester but eventually resulted in forceps delivery of a live male infant at 41 weeks' gestation. The pregnancy and organ transplantation literature is reviewed with special attention, illustrated by the case report, to the physiologic adaptation to pregnancy of the transplanted organ, the potential teratogenic effects of the immunosuppressive drugs used in pregnancy, and the contrasts between renal and other major organ transplants. (Am J Obstet Gynecol 1991;164:1629-34.)

Key words: Organ transplantation, pregnancy

In what was essentially an open letter[1] to bioethicists and philosophers in the January 1990 Hastings Center Report, Leonard Bailey, professor of surgery at Loma Linda University School of Medicine, made a plea for the development of the wisdom of predictive bioethics to replace the reactive bioethics that has surrounded the medical progress of the last two decades. In developing his argument, he described organ transplantation as a contemporary paradigm of medical progress, an idea generated by the desire to help individuals with critical problems, a goal that was initially ahead of its science, but one that has come of age in the last decade, with much improved results but with unresolved societal questions regarding the availability and distribution of resources.

Major organ transplantation is clearly here to stay. Although renal transplantation has been with us for the last two decades, we are now beginning to see young women whose health and reproductive function have been restored by other organ transplants and who are seeking preconception counseling and obstetric care.

This article will briefly describe one such case and then review the current information available to the patient and obstetrician facing the clinical situation with specific reference to renal, heart, and liver transplants.

Case report

A 21-year-old white woman, gravida 3, para 0, was referred for obstetric care at 8 weeks' gestation, 21½ months after orthoptic cardiac transplantation. She had a mitral valve replacement at age 18, followed by a progressive cardiomyopathy resulting in end-stage cardiac disease after 1 year. On referral she was taking 175 mg cyclosporine twice daily, 7.5 mg prednisone daily, 100 mg azathioprine (Imuran, Burroughs-Wellcome Co., Research Triangle Park, N.C.) daily, and 10 mg nifedipine at bedtime. Hematocrit was 34.3%, blood urea nitrogen 28 mg/dl, creatinine 1.1 mg/dl, and cyclosporine level 75 ng/dl. Blood pressure was within the normal range, and the nifedipine was discontinued early in pregnancy. The pregnancy was monitored in a routine manner, with serial ultrasonography demonstrating appropriate growth. Laboratory values were unchanged, apart from a fall in hematocrit to 28% at 30 weeks, and weekly nonstress tests and biophysical profiles showed normal results from 34 weeks' gestation.

Cardiac muscle biopsy at 8 weeks showed mild acute rejection. At 15 weeks there was no evidence of rejection, but mild acute rejection was again noted at 22 and 25 weeks, and moderate acute rejection at 30, 32, and 36 weeks. The rejection was treated by an increase in cyclosporine to 420 mg daily and a minipulse of prednisone. Maximum recorded levels of cyclosporine were

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at 127 ng/dl. At 40 weeks there was no evidence of rejection on cardiac biopsy. A mild bronchospasm developed in early pregnancy that the patient had not previously experienced. This persisted throughout much of the pregnancy and was treated with theophylline.

Labor was induced in the latent phase by artificial rupture of the membranes and oxytocin infusion at 41 weeks' gestation. Progress was normal to 6 cm dilation, but the active phase was then somewhat prolonged. After another 6 hours, labor became arrested with the vertex 5/5 engaged at the +3 station, directly occipitoposterior, with a persistent anterior lip of cervix. The patient had received an epidural anesthetic on entering the active phase of labor. A My-T-Vac vacuum extractor was applied to the fetal head, complete dilation was achieved, and Luikart Simpson forceps was applied, with delivery of a healthy male infant after light traction through three contractions. Infant weight was 2835 kg, and Apgar scores were 9 and 9. Arterial cord gases included pH 7.14, P_{CO_2} 46 mm Hg, P_{O_2} 20 mm Hg, bicarbonate 15 mEq/L, and base excess 15.3 mEq/L. Broad-spectrum antibiotics (vancomycin, gentamicin, and clindamycin) were given at rupture of the membranes and continued into the postpartum period. Obstetric intervention was decided on to limit the risks of infection potentially associated with cesarean section in labor and to limit the effects of prolonged maternal effort associated with a persistent posterior position of the fetal head. The infant, who was not breast-fed, made normal progress in the postpartum period and is now apparently normal, at age 1 year. The patient made normal progress in the immediate postpartum period. She was readmitted to the hospital after 1 month, with an episode of theophylline toxicity. She was discharged after 24 hours. The patient had a laparoscopic sterilization by Falope ring application 6 months post partum. She is now doing well with no signs of rejection.

This patient was not particularly assiduous in seeking preconception counseling, but others have been, and a systematic approach is helpful. Particular consideration should be given to the specific organ transplanted and the general and individual expectations for that transplant, the existence of comorbidity (sometimes the primary condition that led to the transplant), an understanding of the physiologic adaptation of the transplant to pregnancy, an appreciation of the teratogenic and other effects of the immunosuppressive drugs, an appreciation of the potential for organ rejection during pregnancy and how the signs of rejection may be distinguished from other pregnancy considerations, the need to individualize pregnancy management, and, as an amalgam of all the foregoing, the sense of how informed consent is or can be given in any pregnancy.

Transplantation expectations

Cardiac transplants. In the last decade there has been a rapid increase in the number of heart transplant operations performed, with approximately 2500 being reported to the registry of the International Society for Transplantation in 1988.[2] The mean age of patients undergoing transplantation is currently 43.8 years for heart recipients and 29.4 years for heart-lung recipients. Of these, 17% of heart recipients and 59% of heart-lung recipients are female. The 5-year actuarial survival for all orthoptic heart transplant patients was 73.9% and 81.9% for those receiving triple-drug therapy. At 10 years, the survival is 73.3%. By far the greatest mortality appears in the first year.

The major indications for cardiac transplant include coronary artery disease and cardiomyopathy, the latter being more likely to be the indication in young women and unlikely to be associated with significant comorbidity. However, it is important to recognize that 20% of patients will have some degree of allograft vasculopathy.

Hepatic transplants. Long-term survival data for liver transplantation are limited. The United Network of Organ Sharing reports 80% to 85% survival at 1 year and 70% to 75% at 3 to 5 years, with a slightly better survival in women.

Approximately 50% of the patients receiving a liver transplant do so because of chronic active hepatitis. Other indications include alcoholic cirrhosis, sclerosing cholangitis, hepatoma, primary biliary cirrhosis, and fulminant hepatic failure. These conditions are not usually associated with residual morbidity that has a deleterious effect on pregnancy, assuming restoration of normal liver function. Approximately one third of liver recipients are female, 75% of whom are likely to be within the reproductive age group, though most fall toward the upper end. Of 43 patients receiving transplants at Oregon Health Sciences University in the last 2 years, eight were considered to be of childbearing age.

Renal transplants. Evaluation of the circumstances for renal transplant patients differ from heart and liver transplants in two respects. It is possible, of course, for the graft to fail and the patient to survive and the 5-year graft survival varies considerably according to the origin of the graft.

Patient survival after renal transplant is 98% after 1 year, decreasing thereafter by 2% per year. For cadaver grafts survival is 85% for 1 year and 75% for 3 years, and for living related grafts it is 90% for 1 year and 85% for 3 years. In our program 62% of recipients have been male, and 38% female, with the mean age of female recipients being 43 years. While the survival data for renal transplantation are impressive, there remains a high rate of complications, even in those patients with long-term survival. In an article, "Life After Transplantation,"[3] describing the myriad of problems—emotional, financial, physical, and cultural—that transplant recipients experience, one patient states that "transplantation is more a syndrome than a disease because it is actually many diseases. You are trading one disease—the one you had before transplantation—for many others, but these others are treatable." In a study of 57 patients who survived with a functioning allograft for 10 years or longer in a single program in Minnesota[4] and who were studied for a mean of 2.2 years into their second decade, seven patients died, seven had a graft loss, and 40% of patients experienced complications from significant infections (hypertension, hyperlipidemia, liver disease, and musculoskeletal problems). Cataracts developed in one third, one fourth experienced rejection, and 16% had malignancy, vascular occlusive disease, or gastrointestinal disorders. The continuing existence of other conditions that are likely to have an adverse effect on pregnancy is commonplace in renal transplant patients. In our program 33% of patients had insulin-dependent diabetes mellitis, 30% had chronic glomerular nephritis, 10% had polycystic kidney disease, 27% had miscellaneous indications, including infection, obstruction, and congenital defects, as the primary indication for transplantation. The presence of diabetes and hypertension and the possibility of compromised renal function are all likely to be associated with poor pregnancy outcome.

Physiologic adaptation

Cardiac. With only a handful of case reports of pregnancy after cardiac transplantation, there is as yet no body of information describing any changes that occur in the transplanted heart under the influence of pregnancy. Cardiac output is increased in pregnancy by a combination of increase in heart rate and stroke volume caused by an increasing cardiac size. In our patient an increase in cardiac output from the denervated heart was achieved by increasing the heart rate, but cardiac size and contractility were essentially unchanged. A second patient is currently being studied, and the combined results will be reported later.

Renal. The renal adaptation in pregnancy appears to be influenced more by the preexisting graft function than by the fact that the kidney is a graft. When renal function is normal, the glomerular filtration rate increases in the usual way, as shown by the prospective study by Davison[5] of 10 pregnancies, with the greatest increments occurring in those patients whose graft functioned best before conception, regardless of donor source or sex and the transplant-to-pregnancy interval. Some reduction in glomerular filtration rate and increased proteinuria during the third trimester were common and usually transient.

Hepatic. Of the few cases reported of pregnancy after liver transplantation,[6, 7] no particular comments have been made regarding liver function other than a report that liver function "remained normal."

Immunosuppressive therapy

The comment previously made that major organ transplantation's time has come is due in large part to the better understanding of the science of immunology and the use of immunosuppressive drugs. There is now extensive experience with the use of corticosteroids and azathioprine in pregnancy. Corticosteroids have been associated with an increased incidence of cleft lip and palate in animals, yet this has not been seen in humans. The potentially serious effects of maternal hypertension and fetal adrenal insufficiency are probably dose related and are unlikely to be seen at daily doses of <15 mg prednisone. It is impossible to determine whether steroid use plays a role in individual cases of intrauterine growth retardation and premature rupture of the membranes, as has been suggested.

There is now 30 years of experience[8] with azathioprine, the nitroimidazole derivative of 6-mercaptopurine. It is activated by conversion in the liver. Small doses are readily transferred to the fetus, but reported anomalies appear randomly at a rate of about 3%. Uncertainty remains regarding long-term effects, particularly with reference to reproductive potential and risk of malignancy. Chromosomal abnormalities seen in one infant disappeared after 1 year. Leukopenia and thrombocytopenia may occur but are not seen when the leukocyte count is maintained at a normal level. Cyclosporine, a fungal peptide, was first introduced in 1978 and has significantly reduced rejection episodes in cadaver transplants. It crosses the placenta readily and is highly lipid soluble.

Animal studies with doses two to five times the therapeutic level have demonstrated an increased incidence of fetal and neonatal death, growth retardation, and diverse skeletal malformations. One hundred sixteen pregnancies have been reported to the manufacturer (Sandoz Pharmaceuticals, East Hanover, N.J.) with isolated cases of abnormality, none reported more than once, although intrauterine growth retardation was frequently observed.

Pregnancy outcome after renal transplant

Considerable experience[9, 10] has accumulated in the last two decades regarding pregnancy outcome in renal transplants. Initial individual case reports were followed by small cohorts from individual centers by literature review with aggregate reporting and questionnaire surveys. Unfortunately, this sequence has led to an inconsistency in the reporting, so that it is not possible to describe precise outcome figures. In summary, there has been a high therapeutic abortion rate of up to 30%, a high premature birth rate (<37 weeks) of

45% to 60%, a 30% incidence of preeclampsia, and a 20% incidence of intrauterine growth retardation. With the relatively small numbers and the method of reporting, the perinatal death rate is hard to pin down but appears to be of the order of 5%. Pregnancy outcome appears significantly improved if the pregnancy is delayed for at least 2 years after the transplant and if preconception renal function is normal.

One article from South Africa[11] reported 22 live births from 38 pregnancies with seven neonatal deaths. The more successful pregnancies were associated with the absence of warfarin, less immunosuppression, and compliance in taking medication.

Comment

It is now 32 years[12] since the first reported post–renal transplant pregnancy, and a considerable experience has accumulated. It is estimated that one of every 50 women of childbearing age with a functioning transplant will become pregnant. Pregnancies have also been reported after liver, pancreatic,[13] bone marrow,[14] heart-lung, and heart transplants,[15, 16] but these reports, including the case reported here, are single case reports and there is no accumulated experience as yet. The role of the obstetrician as a member of the transplant team has been discussed by Kossoy et al.[17] and presents somewhat of a dilemma. The improvement in survival after major organ transplantation is impressive, and many recipients are able to lead normal lives and resume their employment. It seems churlish to deny a young woman the opportunity of a pregnancy as part of the resumption of a normal life-style. On the other hand, these patients do not lead what for others would be a normal life and do not face a normal life expectancy.

In the ideal situation, the obstetrician will be able to consult with the patient and her family before she makes the decision to conceive. Discussion will include evaluation of the risks of pregnancy and the significance and problems of parenthood in this context. Although there have been individual case reports of maternal death associated with pregnancy after renal transplant, it appears that pregnancy itself carries relatively low immediate risk to the mother. The problems surrounding pregnancy appear to be related to the existence of other morbidity and the potential adverse effects of the immunosuppression rather than the failure of the transplanted organ to cope with the physiologic demands of pregnancy. The significant morbidity experienced by the fetus is largely associated with prematurity and growth, and with the continued improvement in outcome from the neonatal intensive care units, the morbidity is probably largely manageable. The patient then is presented with a choice. It has been calculated[18] that even with improved results there is a 50% chance that a parent with a renal transplant, male or female, will be dead before a child reaches 15 years of age.

These would appear to be poor odds, yet many women have chosen to go ahead and become parents as a result of the restoration of reproductive function and the establishment of normal life. One important question remains unresolved: Can we apply the data accumulated from the experience from renal transplants to the counseling provided to recipients of other organs? Is the generally poor outcome with renal transplants related to the function of the transplanted organ, the immunosuppression, or the coexistence of other disease, or might we expect better results with other organs?

As we enter a new decade, it is hoped that organizations such as the United Network of Organ Sharing may be encouraged to develop a registry of pregnancy outcome so that, along with the ethicists, we may be able to develop our skill as predictive rather than reactive providers.

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Discussion

DR. HOWARD MCQUARRIE, Salt Lake City, Utah. Dr. Kirk's article includes an impressive case report of a post–heart transplantation pregnancy with favorable outcome for both the fetus, which was healthy, and the mother, who did not reject the transplanted heart.

At the University of Utah a heart transplantation program has been operational for 5½ years. Since our results may influence reproductive strategies after heart transplantation in other programs, I am pleased to present a summary and highlights of that program's results (personal communication, Dr. William Gay, Department of Surgery, University of Utah, Salt Lake City). From March 1, 1985, through Aug. 10, 1990, 329 heart transplants have been performed with a 96% 1-year survival rate and an actuarial 5-year survival estimate of 84%. Twelve men have fathered normal children after transplantation, but no women have borne children to date.

Three immunosuppressant drugs—corticosteroids, cyclosporine, and azathioprine—are consistently used in transplantation programs. They all cross the placenta, may affect the fetal immune systems, and, in addition, may be teratogenic. Corticosteroids have the most significant long-term effects in the heart recipient and possibly in the fetus. Some effects are infertility, abnormal bone growth, incomplete sexual maturity, and teratogenesis. Because of these risks, the goal of the transplantation program at the University of Utah is to discontinue steroids by 4 months after transplant. At present, nearly 70% of recipients are free of long-term steroid therapy by that time.

Pregnancy is a marvelously balanced, immunologically neutral state. With immunosuppressant therapy the relationship between the pregnant woman and her fetus is changed and both may have increased risks. Dr. Kirk, please comment on how the mother's immunosuppressant therapy may affect the fetus. Also, please comment on the counterpoint, the risk that lymphocytes or their metabolites that have been transferred from a nonsuppressed fetus may incite a rejection process in the mother.

DR. EMMET J. LAMB, Stanford, California. On Jan. 6, 1968, I arrived early in the morning for gynecologic surgery to find that my case had been bumped by Dr. Norman Shumway's first heart transplantation operation, the first in the United States. Since that time the transplantation team at Stanford has done well over 600 heart transplantations and close to 100 heart-lung or lung transplants (personal communication, Joan Miller, RN, Department of Cardiovascular Surgery, Stanford, Calif.). The cumulative 1 year survival over this period is 86% and the 5-year survival is 62%.

Because of their lower risk of coronary artery disease, young women are represented in the series in smaller numbers than young men, in a ratio of about 2 to 3. I do not know the number who are in the reproductive age group. The transplantation team discourages women from conceiving for the two reasons that Dr. Kirk discussed, the iatrogenic diseases common to any group of transplantation patients and concern about the ability of a denervated heart to compensate for the physiologic changes in pregnancy. Only two of these women became pregnant. One conceived within 2 years after heart transplantation because of peripartum cardiomyopathy; her pregnancy and delivery were uncomplicated. The baby, now 15 months old, and the mother are doing just fine. The other patient, who had transplantation because of a tumor of the heart, also had an uneventful pregnancy and delivery. The baby did well, but the mother died, about 6 months after her delivery, of problems related to the transplantation. This case has been reported.[1,2]

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DR. THOMAS G. MOYERS, San Diego, California. About 2 years ago I had the opportunity to care for a 20-year-old woman with cardiomyopathy who, having undergone cardiac transplantation at Sharp Memorial Hospital in San Diego, had an uncomplicated pregnancy about 2 years later. She had a low-forceps delivery of a near-term fetus in good condition; she did well post partum. In the last year a rejection syndrome has developed and she is being considered for a second transplantation operation. Dr. Kirk, how many pregnancies have been reported in patients who have undergone cardiac transplantation? In my review of the literature I have not found very much on the subject.

DR. KIRK (Closing). Dr. McQuarrie, I do not have any specific recommendation for a strategy to reduce the risk of rejection. From the renal transplantation experience, it does not seem that delivery itself increases the risk of rejection in any obvious way. The 9% rate quoted by Davison[1] in a general review includes a whole variety of sources of information. Immunologists tell me that bromocriptine is an immunosuppressant and that the suppression of lactation is appropriate because it is not recommended that patients breast-feed because of the effects of the drugs on the fetus.

Dr. Lamb, I appreciate your historic vignette. One of the messages that I wanted to get across was that, even though the transplantation teams may be advising their patients not to conceive, patients will conceive. We have to give them as much up-to-date information as we can. The cases you and Dr. Moyers cite again highlight two issues: (1) the need to get a central registry of pregnancies after transplantation and (2) the ethical issues we face all of the time. The focus often is on the 9 months of gestation, whereas the issue really is all that becoming a parent means.

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Transabdominal cervicoisthmic cerclage: A reappraisal 25 years after its introduction

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Most cerclage operations for cervical insufficiency are performed transvaginally. The transabdominal route is beneficial in treating patients with cervices that are either extremely short, congenitally deformed, deeply lacerated, or markedly scarred because of previously failed transvaginal cerclage procedures. The average gestational age at surgery was 11.5 weeks and the operation was performed after early ultrasonographic verification of fetal viability. Patients with advanced cervical effacement or dilatation in the second trimester were excluded. A 5 mm wide Mersilene band was applied in an avascular space above the junction of the cervix and the uterine isthmus without dissection or tunneling among broad ligament vessels. This simplified surgical approach resulted in little operative blood loss (mean, 75 ml; range, 50 to 200 ml). After transabdominal cervicoisthmic cerclage, 21 pregnancies in 20 patients resulted in 18 term births, one premature birth with favorable outcome, and two early fetal deaths (90% salvage rate). A review of the world literature indicated 130 pregnancies with transabdominal cervicoisthmic cerclage during pregnancy and a cumulative success rate of 89%. Preconceptional transabdominal cervicoisthmic cerclage was reported in 30 pregnancies with an overall fetal survival rate of 81%. A survey of specialists in maternal-fetal medicine indicated an increasing interest and familiarity with transabdominal cervicoisthmic cerclage since its introduction more than two decades ago although this procedure is still not widely applied in obstetric practice. (AM J OBSTET GYNECOL 1991;164:1635-42.)

Key words: Cervical defects, incompetent cervix, transabdominal cerclage

Most cerclage operations for cervical insufficiency are performed transvaginally. The transabdominal approach to cerclage placement above the cervix and around the uterine isthmus was originally described by Benson and Durfee[1] in 1965 at the Oregon Health Sciences University. The transabdominal route is beneficial in the treatment of patients with cervices that are either extremely short, congenitally deformed, deeply lacerated, or markedly scarred because of previously failed transvaginal cerclage procedures. The experience with this operation at the same hospital was expanded and updated in 1982.[2]

Since the introduction of transabdominal cervicoisthmic cerclage more than 25 years ago, modifications have been made in patient-selection criteria, surgical technique, timing of the operation, and preoperative and postoperative patient management. In recent years, several reports[3-5] from other centers on transabdominal cerclage techniques have appeared in the literature with uniformly favorable results and few complications reported. In an effort to appraise the significance of these modifications, our experience with transabdominal cervicoisthmic cerclage since 1982 is described. We also conducted a survey of specialists in maternal-fetal medicine to assess their familiarity with transabdominal cerclage techniques. Although transabdominal cervicoisthmic cerclage is still not widely used in obstetric practice, our survey suggests there is an increasing interest and familiarity with this procedure. The place for transabdominal cervicoisthmic cerclage in the management of recurrent second-trimester pregnancy loss and preterm delivery is discussed in the context of our current understanding of the pathophysiology of preterm labor and cervical incompetence.

Patients and methods

Twenty-two patients were selected for transabdominal cervicoisthmic cerclage and 20 patients were followed up through 21 pregnancies in an 8-year period from 1982 to 1990. With the exception of one patient who had transabdominal cervicoisthmic cerclage after extensive conization and near amputation of the cervix, all other patients had prior pregnancy losses in the second trimester or had experienced premature delivery (before 35 weeks' gestation).

The procedure was performed between 10 and 16

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Table I. Clinical indications for transabdominal cervicoisthmic cerclage*

Condition	No. of patients
Marked scarring after failed transvaginal cerclage	18
Abnormally short or deformed cervix	
Diethylstilbestrol exposure	9
Müllerian anomaly	3
Extensive conization	4
Deep forniceal lacerations	5

*Several patients had more than one condition.

weeks' gestation (mean age, 12 weeks; median age, 11.5 weeks) after determination of fetal viability by ultrasonography in 20 patients and before conception in two patients. The patients varied in age from 27 to 40 years. All were multigravid women with a mean gravidity of 5 at the time of the operation. Preoperative patient evaluation included cervical and urine cultures for aerobic and facultative bacteria, *Chlamydia*, and genital mycoplasma organisms. Eight patients received erythromycin, 1 gm orally daily for a minimum of 10 days before surgery for positive cervical cultures for group B streptococci, *Mycoplasma* organisms, or empirically.

Eighteen patients had at least one previously failed transvaginal cerclage; eight patients had two failed MacDonald or Shirodkar procedures before transabdominal cervicoisthmic cerclage. The clinical selection criteria for transabdominal cervicoisthmic cerclage are listed in Table I. Several patients had more than one indication. All patients had abnormally short, markedly scarred, or deeply lacerated cervices. Nine patients gave a history of exposure to diethylstilbestrol in utero and all demonstrated short cervices and previously failed transvaginal cerclage procedures. Histories of predisposing or contributory factors such as obstetric or surgical trauma to the cervix were elicited from nine patients. Four patients had extensive cervical lacerations after traumatic or precipitous delivery or a uterine rupture. Four patients had extensive conization of the cervix because of cervical intraepithelial neoplasia and two patients had virtual amputation of the cervix after conization had been complicated by bleeding. Two patients had term deliveries before an extensive cervical laceration or wide conization, which in turn were followed by second-trimester fetal death. Three patients had deformed cervices in association with Müllerian anomalies. One patient had unicornuate uterus, one had a bicornuate uterus with rudimentary horn and double cervix, and another patient demonstrated vaginal agenesis and partial cervical atresia that had been treated by a modified McIndoe procedure. Transabdominal cervicoisthmic cerclage was placed as an interval procedure before conception in two patients, in one case at the patient's request and in the other case because the lower uterine segment was severely scarred from a previous uterine rupture.

Surgical procedures. Fourteen procedures were completed after induction of general anesthesia and seven after induction of regional anesthesia. Twenty operations were performed during pregnancy and two before conception. The peritoneal cavity was entered by a Pfannenstiel incision in 14 patients and by midline vertical abdominal incision in eight patients with a previous scar with known pelvic adhesions, or when difficult surgical exposure was anticipated.

Between 1982 and 1985, transabdominal cervicoisthmic cerclage was performed as previously described[2] in nine patients (group 1). After dividing the peritoneal reflection and advancing the bladder, the space between the ascending and descending branches of the uterine arteries was identified lateral to the uterine isthmus and a tunnel was developed by blunt dissection. After tenting the posterior leaf of the broad ligament with right-angle forceps, it was incised with Metzenbaum scissors and a 15 cm segment of Mersilene ribbon (0.5 cm wide, Mersilene 5, RS21, Ethicon, Inc.) without needles was passed under direct vision. An identical procedure was carried out on the opposite side. The Mersilene band was secured anteriorly with a triple square knot and the cut ends were fixed to the band with nonabsorbable sutures.

Our current and preferred technique was adopted after 1985 and was used in the other 13 patients (group 2). It is a modification of Mahran's technique[6] but with several important differences. After the lower uterine segment is exposed, the uterine vessels are located by palpation between the thumb and the forefinger and are drawn laterally away from the uterine isthmus. Preoperative planning and coordination between the surgeon and the assistants is essential to provide maximum exposure and to avoid injury to the dilated blood vessels. Elevation of the uterus out of the pelvis by the assistant is a critical step because it allows visualization of the uterine vessels, the isthmus, and the uterosacral ligaments. If the surgeon is right-handed, the 5 mm Mersilene band with swaged needle (RS21 with blunt tip) is passed in a posterior direction (on the patient's right side) through an avascular space between the retracted vessels and the uterine isthmus without penetrating the myometrium. The suture is passed above the uterosacral ligaments. The same procedure is performed on the opposite side but the needle enters from behind the uterus in an anterior direction. The band should lie flat and snugly and the knot is tied anteriorly with firm compression of the intervening tissue. Handheld retractors are preferable to self-retained retractors because they provide better exposure and more room to maneuver the uterus.

After the operation, the patient is monitored for blood loss and fetal viability. Uterine irritability is rare unless the cervix is effaced or dilated.[7] Prophylactic hormones, tocolytic agents, or antibiotics are not routinely administered after the operation. All patients were discharged within 4 to 7 days after transabdominal cervicoisthmic cerclage and the condition of the cervix was assessed by palpation at weekly or biweekly intervals. Cervical length and status were further assessed by transabdominal or transvaginal ultrasonography at periodic intervals.

Results

All patients have been followed up to the time of this writing. Of the 22 patients, four have elected to have a tubal sterilization procedure, two have had a hysterectomy, and 16 remain voluntarily fertile.

Fetal survival. The obstetric outcomes of 21 pregnancies in 20 patients who had transabdominal cervicoisthmic cerclage during pregnancy are tabulated in Table II and compared with the results in the same patients before cerclage. The two patients who had transabdominal cervicoisthmic cerclage in the nonpregnant state had not conceived after 6 to 12 months.

The overall fetal survival rate in patients after transabdominal cervicoisthmic cerclage was 90%; that before cerclage was 20% ($p < 0.01$). Of the 20 patients, one had two successful pregnancies and repeat cesarean section deliveries after the transabdominal cerclage.

The effect of transabdominal cervicoisthmic cerclage on the distribution of births by gestational age is shown in Table III. Seventy percent of the pregnancies before cerclage ended in late abortion. Five percent of the postcerclage pregnancies also did so. Likewise, 18% of the precerclage births but only 5% of the postcerclage births were premature (at ≤35 weeks' gestation). Ninety percent of the deliveries after transabdominal cervicoisthmic cerclage but only 12% of those before transabdominal cervicoisthmic cerclage resulted in live births at term. Two patients had term deliveries before an extensive cervical laceration or wide conization that led to subsequent second-trimester fetal death. Successful term deliveries were once again achieved after transabdominal cervicoisthmic cerclage in these two patients. The differences in the distribution of late abortions, premature deliveries, and term deliveries were highly significant statistically (χ^2, $p < 0.001$). All infants were delivered by cesarean section. A transverse incision was made in the lower uterine segment but the band was not disturbed.

Complications. In this series of 22 patients, 20 experienced no intraoperative or postoperative complications (i.e., no hemorrhage, sepsis, fever, premature rupture of the membranes, or premature labor). The majority of patients received prophylactic antibiotics in the operating room. In two patients, fetal death in utero occurred after transabdominal cervicoisthmic cerclage at 13 and 14 weeks' gestation, respectively. In one patient, transabdominal cervicoisthmic cerclage was placed uneventfully at 11 weeks' gestation after preoperative ultrasonography had shown a vanishing twin syndrome. Fetal heart tones disappeared without apparent cause at 13 weeks' gestation and the products of conception were evacuated without difficulty with the band in place. Chromosomal studies on the products of conception showed no abnormalities.

In the other patient, laceration of a uterine vein occurred during the blunt dissection and tunneling among broad ligament vessels. This led to an estimated blood loss of 450 ml. Hemostasis was achieved by the application of clips and by suture ligature. Fetal heart tones were lost on the sixth postoperative day, accompanied by vaginal bleeding. The fetus and products of conception were removed by cervical dilatation and curettage without removing the Mersilene band. Two days later, acute pelvic thrombophlebitis developed that required heparin and antibiotic therapy. The subsequent recovery was uneventful.

The average estimated operative blood loss differed significantly between patients who had placement of the cerclage by tunneling between uterine vessels

Table II. Obstetric outcome in 20 patients before and after transabdominal cervicoisthmic cerclage*

	Before cerclage	*After cerclage*
Ectopic pregnancy	3	0
Early abortion	18	1
Late abortion	34	1
Premature delivery		
With neonatal survival	4	1
With neonatal death	5	0
Term delivery	6	18
Total number of pregnancies	70	21
Fetal salvage rate (%)†	20	90

*Excludes two patients with preconceptional cerclage.
†Excludes early abortions; χ^2, $p < 0.01$.

Table III. Effect of transabdominal cervicoisthmic cerclage on the distribution of births

Gestational age at delivery (wk)	*Before cerclage*		*After cerclage*	
	No.	*%*	*No.*	*%**
14-23	34	70	1	5
24-35	9	18	1	5
≥36	6	12	18	90

*χ^2, $p < 0.001$.

Table IV. Comparison of results with two surgical techniques for transabdominal cervicoisthmic cerclage

	Group 1 (n = 9)	*Group 2 (n = 11)*
Surgical technique	Tunneling between vessels	Manual retraction of vessels
Gestational age at cerclage (wk)	13 (11-16)	11 (10-14)
Estimated blood loss (ml)*	250 (75-450)	75 (50-200)
Fetal salvage rate (%)	89	91

*Data represent mean with range in parentheses; $p < 0.05$.

Table V. Responses to survey of perinatal obstetricians

	No.	*%*
Respondents	248	57
Perform TCIC	86	35
TCIC procedures	404	—
Before pregnancy	43	11
First trimester	112	28
Second trimester	249	61
Fetal survival rate	—	87
Premature births	118	25
Term births	360	75
Use perioperative adjunctive therapy		
Antibiotics		20
Tocolytic agents		44
Prostaglandin inhibitors		22
Obtain preoperative cervical cultures		65

TCIC, Transabdominal cervicoisthmic cerclage.

(group 1) and those in whom uterine vessels were retracted manually (group 2). The latter procedure was adopted after 1985 and resulted in substantially less blood loss (Table IV).

Late prenatal complications after transabdominal cervicoisthmic cerclage consisted of premature labor in three patients (an incidence of about 14%) who responded to the administration of tocolytic agents. One patient experienced placenta accreta at 39 weeks' gestation, resulting in hemorrhage, shock, coagulopathy, and cesarean hysterectomy.

Questionnaire. We sent questionnaires to 435 members of the Society of Perinatal Obstetricians and 248 were returned (57%) (Table V). Eighty-six of the respondents (35%) stated they had personal experience with transabdominal cerclage and had performed between 1 and 25 procedures. Only ten obstetricians had performed more than 10 such procedures. Ninety-six percent of the respondents were familiar with the operation and 90% stated that transabdominal cerclage had a role albeit limited in comtemporary obstetric practice. A total of 404 transabdominal cerclage procedures were tabulated at the time of this writing, which is approximately three times the number reported in the world literature. Most (61%) were performed in the early phase of the second trimester of pregnancy and only 11% were done before pregnancy. Seventy-five percent of pregnancies resulted in term births and there was an overall uncorrected fetal survival rate of 87%.

Comment

Injuries to the cervix during childbirth or after extensive conization are major causes of traumatic cervical incompetence and result in a high rate of second-trimester abortion and premature delivery.[1, 8] In our series, cervical incompetence was attributed to surgical trauma (extensive cervical conization) in four patients and to deep forniceal lacerations in five others. In two patients, the cervix and lower uterine segment were lacerated and deformed as a result of a previous uterine rupture or extension of a transverse incision at cesarean section. In nine patients, the cervix was congenitally short (<1 cm or flush with the vagina) in association with diethylstilbestrol exposure in utero.

Cervical insufficiency may coexist with müllerian anomalies. One of our patients had a unicornuate uterus, another had a bicornuate uterus with rudimentary horn and double cervix, and a third patient had vaginal agenesis with cervical atresia. There is increasing evidence that cervical cerclage improves obstetric outcome in pregnancies complicated by uterine malformations,[9] although most gynecologists prefer a septum resection or metroplasty for the uncomplicated septate or bicornuate uterus.[10] Cerclage may be the only option for the patient who is delivered of an infant prematurely in association with the unicornuate uterus. We report a successful pregnancy after transabdominal isthmic cerclage in a patient with vaginal agenesis and cervical atresia treated by a modified McIndoe procedure.

The primary clinical considerations in choosing a transabdominal cerclage rather than a transvaginal procedure were marked scarring of the cervix and previously failed transvaginal cerclage procedures in 18 patients (82%) (Table I). In contrast to a previous report,[2] we did not perform emergency transabdominal cervicoisthmic cerclage for the patient with advanced cervical effacement or dilatation in the second trimester. Others,[3, 5] have reported a high incidence of premature rupture of the membranes under these conditions. Also, our recent success in the management of patients with a previable fetus and bulging membranes in the absence of symptomatic contractions would suggest that a combination of transvaginal Shirodkar cerclage, broad-spectrum antibiotic therapy, tocolysis, and inhibition of prostaglandin synthesis is preferable to transabdominal cervicoisthmic cerclage.[11]

Table VI. Cumulative results of transabdominal cervicoisthmic cerclage performed during pregnancy*

Authors	*Year*	*No. of patients*	*No. of pregnancies*	*Fetal survival (%)*	
				Before TCIC	*After TCIC*
Benson and Durfee[1]	1965	10	13	11	82
Watkins[17]	1972	2	2	55	100
Mahran[6]	1978	10	10	10	70
Olsen and Tobiassen[15]	1982	17	17	12	88
Novy[2]	1982	16	22	24	95
Wallenburg and Lotgering[4]	1987	14	16	16	94
Herron and Parer[3]	1988	8	13	15	85
van Dongen and Nijhuis[5]	1990	14	16	30	94
Novy (this series)	1990	20	21	20	90
TOTAL, mean (%)		111	130	21	89

TCIC, Transabdominal cervicoisthmic cerclage.

*Includes two patients with preconceptional cerclage.

Table VII. Cumulative results of transabdominal cervicoisthmic cerclage performed before pregnancy

Author	*Year*	*No. of patients*	*No. of pregnancies*	*Fetal survival (%)*	
				Before TCIC	*After TCIC*
Ardillo[16]	1969	11	7	1	29
Loock[18]	1980	3	3	10	100
Olsen and Tobiassen[15]	1982	12	19	12	95
Marx[19]	1989	1	1	29	100
TOTAL, mean (%)		27	30	13	81

TCIC, Transabdominal cervicoisthmic cerclage.

Fetal salvage rate after transabdominal cervicoisthmic cerclage in the series reported here was 90%. The precerclage rate for the same group of patients was 20% ($p < 0.01$). The cumulative results in the world literature of transabdominal cervicoisthmic cerclage performed during pregnancy are shown in Table VI. Since the original report by Benson and Durfee in 1965, there have been eight reports from other centers including this series. A total of 130 pregnancies have been followed up in 111 patients with an average fetal salvage rate of 89%. A smaller number of patients has been followed up after transabdominal cerclage performed before conception. Table VII indicates that a similar fetal salvage rate of 81% was achieved in 30 pregnancies. The consistent success of the transabdominal cerclage over more than two decades and in many different centers in this country and abroad is impressive. It surpasses the overall success rates reported for transvaginal treatments of the incompetent cervical os syndrome.[12] The success rate of transabdominal cervicoisthmic cerclage is especially noteworthy in view of the severe obstetric histories and the failures of transvaginal cerclage procedures in these patients. The superior results with transabdominal cervicoisthmic cerclage in contrast to transvaginal procedures may be explained in part by the specific criteria for cervical insufficiency in this selected group of patients and the higher anatomic placement of the cerclage.

The etiologic factors involved with the incompetent cervical os syndrome are commonly attributed to acquired causes (usually traumatic), congenital factors (e.g., müllerian malformations, or diethylstilbestrol exposure in utero), or functional causes (endocrine factors or infection). Clearly patients who have transabdominal cervicoisthmic cerclage are selected on the basis of anatomic or acquired cervical defects and thus represent a pure subpopulation of patients in comparison with the heterogeneous group that receives transvaginal cerclages. Even so, a substantial number of patients report a history of premature labor, premature rupture of the membranes, and chorioamnionitis in prior pregnancies but infrequently after transabdominal cervicoisthmic cerclage. There is increasing evidence that cervical or vaginal microflora, or the inflammatory reactions they produce, release factors (e.g., proteases, collagenases, prostaglandins, and lymphokines) that initiate or predispose the patient to uterine contractions, cervical ripening, and rupture of the membranes.[13, 14] Although speculative, the possibility exists that anatomic defects in the cervix predispose the patient to ascending lower genital tract infection and that in some way transabdominal cerclage procedures

Table VIII. Complications associated with transabdominal cerclage

Complication	*Incidence*	*References*
Excessive blood loss	Occasional	1,2,3,5
Fetal death, abortion	Occasional	1,2,5,6,15
Intrauterine growth retardation	Occasional	1,3,15
Rupture of membranes	Occasional	1,3,5
Thrombophlebitis	Rare	This series
Placental abruption	Rare	1,5
Bladder discomfort	Rare	4
Persistent pain	Rare	6,18
Suture migration	Rare	2,5

bolster maternal host defenses to ascending infection by restoring the integrity of the cervical barrier during pregnancy.

Despite the efficacy of transabdominal cervicoisthmic cerclage in a selected group of patients, some researchers believe it has several disadvantages (i.e., the need for two intraabdominal procedures, surgical intervention in a highly vascular area, and a moderate complication rate).[12] The need for two abdominal operations during pregnancy cannot be denied but we have experienced few major operative complications and little maternal morbidity with transabdominal cervicoisthmic cerclage. Pelvic thrombophlebitis developed in one patient but there were no cases of maternal infection, premature rupture of the membranes, or chorioamnionitis in this series. Preterm labor occurred in three patients (14%) during the third trimester and was managed successfully with tocolytic agents. Fetal death, intrauterine growth retardation, suture migration, premature rupture of membranes, placental abruption, and excessive blood loss have been reported in the literature but they occur infrequently (Table VIII).[3-5]

In an attempt to avoid additional surgery during pregnancy and to circumvent operative blood loss, some researchers have recommended that transabdominal cervicoisthmic cerclage be performed as an interval procedure in the nonpregnant state. There is still insufficient experience with preconceptional cerclage to conclude that it is superior or even equivalent to operation during pregnancy. Moreover, several potential disadvantages to the performance of cerclage before conception have been reported. It may increase the difficulty of spontaneous abortions or contribute to dysmenorrhea or infertility.[15, 16] We prefer postconceptional transabdominal cervicoisthmic cerclage application because fetal viability can be confirmed and the tension applied to the Mersilene band is more accurately judged during pregnancy. A modification in our surgical technique has led to a significant reduction in operative blood loss and has virtually eliminated serious bleeding complications during surgery. The use of ultrasonography to determine fetal viability at 9 to 10 weeks' gestation has in turn allowed surgical intervention earlier in the first trimester of pregnancy. Earlier timing of the operation (at approximately 11 weeks' gestation) facilitates the surgery and reduces intraoperative complications.

In the 25 years since its introduction, there has been progressive worldwide use of transabdominal cervicoisthmic cerclage although it remains a specialized procedure. The results of our survey of perinatal obstetricians in the United States indicates that about one third have performed the procedure in their own practices. Nevertheless, a broad consensus was obtained that transabdominal cervicoisthmic cerclage is an established procedure that is tailored to a select group of patients with anatomic defects of the cervix and failed transvaginal cerclage. Because transabdominal cervicoisthmic cerclage is recognized as an effective and safe operation, it is unlikely that a prospective trial will ever be mounted. There is even serious concern that such a trial would be ethical. Moreover, a sufficient number of women have been described in the world literature who had normal term deliveries but after a distinct traumatic event (e.g., extensive conization) experienced repeated fetal death. The pattern of pregnancy wastage was then restored to normal by transabdominal cervicoisthmic cerclage. This sequence of events is analogous to the therapeutic response after other curative surgical procedures and is highly unlikely to be a chance phenomenon.

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Discussion

DR. LYMAN RUST, Hanford, California. Dr. Novy has just presented his second article before this society describing his experiences with transabdominal cervicoisthmic cerclage. I reviewed this article with a great-deal of curiosity and interest because I have had no personal experience with this procedure. And, indeed, I have never been able to determine the indications for the transabdominal approach when the transvaginal procedures have served so well.

Dr. Novy discusses a survey of perinatologists of whom only 35% of the respondents had had personal experience with transabdominal cervicoisthmic cerclage. I would venture to say if a similar survey were taken of obstetricians who do not label themselves as perinatologists, this figure would be considerably lower. There are several important reasons for the lack of enthusiasm for this approach. First, most obstetricians have had excellent results with the classic transvaginal procedures developed by Shirodkar and MacDonald. Second, the transabdominal cervicoisthmic cerclage procedure has much greater morbidity, requires a second laparotomy for cesarean section, and is thus much more expensive. And third, the indications for transabdominal cervicoisthmic cerclage are strictly limited.

It is the latter point, the indications for transabdominal cervicoisthmic cerclage, that is the most important contribution of this article. Dr. Novy has selected his patients very carefully with well-defined criteria. However, with the obvious drawbacks of this procedure, these criteria need to be carefully scrutinized. As reported in the article, all 22 patients in this series had well-defined cervical deformity and 21 had previous pregnancy losses in the second trimester or premature delivery before 35 weeks' gestation. Eighteen of the 22 had a previous unsuccessful attempt at transvaginal cerclage and eight patients had two previous failures. That sounds like very strict criteria. However, I would emphasize that failure of a transvaginal cerclage per se is not necessarily an indication for the abdominal approach. Other causes of failure such as the indication for the initial cerclage, ruptured membranes, or bleeding should be considered and ruled out. Occasionally a cerclage stitch will tear out because of improper placement or because of the type of suture used. A No. 1 or No. 2 suture might cut through the cervix where a wide band (5 mm) Mersiline suture might not.

Also, patients with a short, scarred or deformed cervix can and have been successfully treated with transvaginal cerclage. A technique described by Jesse Rust with the use of a curved Allis clamp to grasp and gather the side of the cervix plus the gentle insertion of the index finger into the cervical canal to identify the internal os, allows for the safe placement of a deep stitch at the level of the internal os in most instances.

Therefore I would agree with Dr. Novy that the indications for transabdominal cervicoisthmic cerclage should be strictly limited to those patients who have a marked cervical deformity and who have failed a previously well-placed transvaginal cerclage.

Indications for use of transabdominal cervicoisthmic cerclage for patients with müllerian anomalies and women exposed to diethylstilbestrol still need verification. It is not clear from this article whether the three patients in this category had pregnancy losses consistant with cervical incompetance and whether they benefited from transabdominal cervicoisthmic cerclage.

Dr. Novy is to be congratulated on his perseverance with this procedure. He has developed modifications in surgical technique to lessen the risk of blood loss. However, performance of transabdominal cervicoisthmic cerclage in the first trimester needs to be questioned. Although ultrasonography allows early detection of fetal heart rate, two of his patients suffered loss of viability soon after surgery was performed. Maybe these losses resulted from the surgery or possibly they were doomed regardless. Most obstetricians believe that elective surgery is better performed in the second trimester after the pregnancy is well established and the uterus is more quiescent.

In closing, Dr. Novy is to be commended for his continued interest in and modifications of transabdominal cervicoisthmic cerclage and for clarification of the strict indications for this procedure. His results speak for themselves: transabdominal cervicoisthmic cerclage does work.

I have these questions for Dr. Novy:

1. Why were prophylactic antibiotics used? Do you have any controlled studies that show they are advantageous?
2. Did the patients with müllarian deformities have previous pregnancy losses consistant with incompetent cervix or were they done empericaly?

3. Were chromosomal studies done on the two fetuses that died after transabdominal cervicoisthmic cerclage.

Dr. Novy (Closing). Failure of a previous transvaginal cerclage should not be the sole indication for a transabdominal procedure. On several occasions patients have been referred to me for a transabdominal cerclage, but on examination I found that a transvaginal Shirodkar cerclage could be applied and it was done successfully with a good outcome. The decision to apply a transabdominal cerclage should be made on the basis of the obstetric history, the details of previous attempts at transvaginal procedures, and the current anatomic state of the cervix. A significant deformity, a laceration, extreme shortening, or severe scarring may make the transvaginal approach difficult.

Concern about operating in the first trimester of pregnancy is less today than it was before ultrasonography. We have ample evidence from studies relating to chorionic villus biopsy[1] that an ultrasonographic diagnosis of fetal viability at eight weeks' gestation is associated with a subsequent pregnancy loss of only 3%.[1] Therefore ultrasonography-proved viability between 8 to 10 weeks' gestation allows us to intervene well before the traditional 13 or 14 weeks' gestation and to do so with a 95% likelihood that the pregnancy will be maintained, barring a complication of surgery. Operation at 11 weeks' gestation is associated with less operative blood loss and can be done through a Pfannenstiel incision, which is a major advantage for the patient. At 14 or 15 weeks' gestation a vertical or transverse muscle-splitting incision is required for exposure.

We used prophylactic antibiotics, a single dose of a cephalosporin preoperatively, in addition to any earlier treatment of abnormal cervical cultures.

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Improved mammographic accuracy

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Use of ancillary imaging studies to evaluate suspicious screening mammograms can improve their positive predictive value for detecting malignant lesions. In 1989 8181 screening mammograms were performed at the breast evaluation center at Memorial Hospital Medical Center in Long Beach. A total of 670 patients were called back for magnification views or ultrasonographic evaluation, and 92 of these patients underwent surgical biopsy. There were 42 cancers diagnosed, yielding an accuracy rate of 46%. (AM J OBSTET GYNECOL 1991;164:1643-6.)

Key words: Screening mammography, magnification views, ultrasonography positive predictive value

The lifesaving benefits of screening mammography have been convincingly demonstrated.[1] The American College of Obstetricians and Gynecologists, the American Cancer Society, and other professional and lay groups have established guidelines for the use of this procedure.

Surveys have shown that whereas the majority of primary care physicians are aware of the guidelines and benefits of mammography, only a minority of them recommend routine mammography to their patients.[2,3] A recent survey by the National Cancer Institute demonstrated that only 25% to 41% of women over the age of 50 had had a mammogram during the previous year. One of the chief reasons for not having the procedure done was that the patient's physician did not recommend it.[3]

One of the major reasons cited by physicians for not ordering screening mammography is the cost of the procedure.[4] This refers not only to the charge for the test itself but also to the charge for the large number of additional procedures and biopsies generated by mammography, which has, until recently, had a relatively low rate of accuracy in detecting malignancy.

Reports in the literature state that the positive predictive value of mammography for malignancy ranges from 10% to 30%. A presentation by Dr. Goodno to this society last year indicated that in his community the diagnostic accuracy was approximately 14%.[5] Before the establishment of the breast center in Long Beach, the malignancy detection rate was 12%.

Methods

In 1987 a breast evaluation center was established as part of the Memorial Cancer Institute at Long Beach Memorial Medical Center. The goal was to be able to thoroughly evaluate patients with imaging modalities and other ancillary procedures at one facility.

We use dedicated film screen mammographic equipment with the ability to perform magnification views. Ultrasonographic equipment is available on site to help differentiate solid from cystic lesions. The technicians performing the procedures have received extensive training in mammography and breast ultrasonographic examinations. There is a full-time, experienced radiologist at the center who, in most cases, determines what ancillary procedures need to be performed once the patient has been examined and the mammograms reviewed.

In addition to these imaging modalities, fine needle aspiration is available. A pathologist is present to evaluate aspiration of palpable solid lesions to be certain that sufficient tissue is obtained to make a diagnosis.

Symptomatic women who are referred to the breast evaluation center because of pain, suspected mass, bloody nipple discharge, or other reasons have whatever diagnostic modalities are believed necessary, including fine needle aspiration.

Screening mammograms are also performed at the breast evaluation center. An examination consisting of a craniocaudal and a mediolateral oblique view is performed with film screen radiography. At the suggestion of Dr. Laszlo Tabar,[6] a protocol was established for the evaluation of patients with suspicious or equivocal findings on screening films. Instead of recommending surgical biopsy, we ask these patients to return for additional imaging procedures. Magnification views are used to evaluate architectural distortion or suspicious calcifications. Ultrasonographic studies are performed to differentiate solid from cystic nodular lesions seen

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on the screening films. Other imaging modalities such as thermography, light scans, or computerized tomography have not been used to evaluate these patients.

Results

In 1989 8181 screening mammograms were performed. Of these, 670 patients were called back for additional imaging studies. Surgical biopsy was recommended in 92 of these patients. Biopsy was performed with fine needle localization, since no palpable abnormalities could be detected. There were 42 cancers diagnosed, yielding an accuracy rate of 46%.

Comment

With the advanced technology and training available to radiologists interested in mammography, the ability to evaluate suspicious findings has been vastly improved by the addition of ancillary imaging studies, such as magnification views and ultrasonography. While these add a slight additional charge, the majority of confusing findings on screening mammograms can be more adequately evaluated. This results in a more confident radiographic diagnosis that frequently precludes the need for surgical biopsy.

It has been a European philosophy for years, advocated by Dr. Tabar,[6] that no patient should undergo operation on the basis of a two-view routine screening mammogram. The screening mammogram is only a preliminary survey of the patient. He stated that abnormalities seen on a screening study need additional imaging to determine whether they represent actual abnormality, overlapping artifacts, or classically benign abnormalities.

Improved diagnostic accuracy of mammography results in impressive cost savings. In this study approximately 8% of the patients screened were asked to return for magnification views or ultrasonographic studies. Since the additional charge for these evaluations is $65, the extra charge for the 670 patients was $39,455. Charges for a breast biopsy in our area total about $3000. The costs for the 92 patients who underwent biopsy ($276,000) plus the charges for the additional imaging studies for 670 patients total $315,455. Had biopsy been the procedure of choice to evaluate the suspicious screening radiographic findings, it would have been much more expensive, both financially and in the emotional and psychosocial stress for the patients.

Additional imaging studies were used more liberally than surgical biopsy would have been, but if we assume a 12% malignancy rate at our facility before the present protocol, approximately 350 women would have undergone a biopsy at a total cost of $1,050,000. Clearly, the use of high-quality imaging in the hands of experienced radiologists results in tremendous savings.

In conclusion, the positive predictive value of routine screening mammograms can be greatly enhanced when patients with equivocal findings are studied further with magnification views or ultrasonography. Reducing the number of unnecessary biopsies will not only lower costs but may encourage more primary care physicians to recommend this valuable cancer screening modality to their patients. Clearly, as when any consultant is used by primary care physicians, careful attention to the radiologist's experience and the quality of the equipment used is an important variable when patients are referred for these evaluations.

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Discussion

DR. KEITH P. RUSSELL, Los Angeles, California. Dr. Pillsbury, how many of the mammograms were ordered by obstetrician-gynecologists? Members of our specialty have almost quadrupled their use of mammographies within the past 10 years. It is interesting that general surgeons, who consider treatment of the breast their domain, sometimes excessively in my opinion, are at the bottom of the list as far as ordering mammograms.

The information you presented may be deceptive unless it is expanded. You presented the results of >8000 mammographies and 92 biopsies, 42 of which were positive for cancer. The implication is that these 42 were the only positive results among the approximately 8000 women. However, there must have been some missed breast cancers in the women who did not have a biopsy. Although I agree that we should not be rushed into doing biopsies, I need to know if there were any missed cancers in the follow-up of those other women? The critical issue is how many breast cancers were missed. It is not enough to say that a smaller number of women underwent biopsy, and thereby we saved many dollars.

Some of the stated resistance by physicians to ordering mammography is the cost. Dr. Pillsbury, I know this unit is not in your office, but I would like to know the cost of an initial screening mammogram. Has your group considered distributing the cost of the additional workup over all of the population served and adding a small amount, perhaps $5, to the initial charge so that there would be no additional charge?

Dr. Gilbert A. Webb, San Francisco, California. I also wanted to express my concern about the lack of information on the false-negative rate of the magnification views and the ultrasonography. If you do not have the data, can you tell us what is the rate reported in the literature? In addition, can you tell us the percentage of invasive cancers versus in situ cancers in the biopsies of your cases? How many were lobular, and how many were ductal?

Dr. William Hindle, Los Angeles, California. Dr. Webb, in the screening mammography program at the Massachusetts General Hospital,[1] 35% of the diagnosed carcinomas were in situ. Before the screening program the rate was about 5%. Of course, most of those are ductal carcinomas.

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Dr. John A. Goodno, Arcadia, California. Dr. Pillsbury, you are to be commended for using a dedicated radiologist to read all the mammograms in the breast center you established. I hope that other gynecologists will establish community breast centers.

Dr. Ronald P. Millard, Bellingham, Washington. A radiologist who limits his practice to mammography asked if we would be interested in having his technician and equipment come into our office once a week to offer mammography. We agreed to try it because both the radiologist and his equipment have good reputations and the charge is only $65 for the screening and $20 for any follow-up. I believe that compliance on screening mammography has been virtually 100%. Our patients are delighted; they are more comfortable in their gynecologist's office than in a radiologist's office to which they had never before gone.

Dr. J. Paul Shively, San Francisco, California. A van from the radiology department of the University of California, San Francisco, goes out on a schedule to various neighborhoods. The cost for routine mammography is low, initially $30 and currently $50, and the service is magnificent. They telephone our office the next day to report any suspicious screening film, and they report the need for magnification imaging, ultrasonography, or biopsy.

Dr. Charles F. Langmade, Pasadena, California. You are very fortunate to have the same radiologist study all of your mammograms. Many of us get reports from several radiologists, depending on where the patient lives. Do you have any suggestions for dealing with the problem of the defensive medicine practiced by some radiologists who overinterpret mammograms and pathologists who overinterpret the slides?

Dr. Pillsbury (Closing). Dr. Russell, I do not have data on how many mammograms were ordered by obstetrician-gynecologists, but I suspect most of them were.

The current charge for a screening mammogram at the breast evaluation center is $95. To minimize the cost of the procedure, the screening is performed by technologists, and the films are read in a batch later the same day or the next day. Thus women with suspicious findings must be called back. The extra charge for either an ultrasonographic examination or a magnification view is $65. It is a provocative suggestion to include the cost of these additional tests required by about 8% of women (670/8180) in the charge for the screening mammogram, but I do not know whether that is possible with the present systems of reimbursement.

With any screening test one must be concerned about both sensitivity and specificity. One wants a screening test to be very sensitive so it doesn't miss any abnormalities. It is about the sensitivity of our protocol that both you and Dr. Webb expressed concern. You asked how many of the 670 women with suspicious mammograms would have had cancers not diagnosed by the management strategy we used had all of them had a biopsy. I do not have the data on the rate of false-negative results with this protocol, since not enough time has gone by to detect cancers that may have been missed. One encouraging fact is that the total number of cancers detected is what one would expect from a comparable group. Among 1000 women over the age of 50 not screened before, you should expect to find seven cancers.[1] Among previously screened women the rate is 3 or 3.5. We detected 42 cancers in a group of 8180 women, some of whom had previously been screened, for a rate of about 5 per 1000, which falls within that range.

Dr. Goodno and Dr. Millard, I believe the trend toward performing mammograms in the gynecologist's office is a good one. Anything we can do to make the procedure more convenient will enhance compliance. For a number of years I have found that many patients do not get the mammograms I order. Just as in the television commercials, they say, "I didn't have time." Now I can say, "Just go right across the hall and get it now." I think this is a tremendous help for patients. We need to be certain, however, that the films taken are of high quality and are read by experienced mammographers. In our office we now have a machine and a full-time technologist. In the interest of quality control, we develop the film and then have a radiologist interpret it. We charge $100, $5 more than the screening center charges.

Dr. Shively mentioned another way to increase compliance. If we are truly concerned about the health care of women, we should be alarmed by the knowledge that 60% to 80% of eligible women do not get screening mammography. We know that 7 of 1000 women who have never been screened have breast cancer. Let us try to encourage patients to get regular screening mammograms. We can detect early malignancies at a time when the vast majority of the patients can be cured.

Dr. Langmade, we need to encourage our radiologists to be true consultants, sharing the responsibility for making a definite diagnosis. Screening for breast cancer is a team effort. In private practice we direct patients to our consultants. We find radiologists who will give us meaningful reports and act as true consultants. There are some mammographers whom we never use because their reports are full of equivocations and disclaimers. They try to make the responsibility all ours. We encourage our mammographer to state that the mammogram is normal and needs to be repeated only at routine intervals or, if it is abnormal, to state which additional study or biopsy is indicated. This is the mark of a real consultant.

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Conservative management of breast fibroadenomas

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Is it conservative or radical management to excise all fibroadenomas of the breast, especially in women <30 years old? Once a definite diagnosis is established by physical examination, fine-needle aspiration cytologic testing, and mammography, is it prudent to monitor women with small fibroadenomas (<4 cm in diameter)? We reviewed 498 cases of biopsy-proved fibroadenomas and 17 cases of phyllodes tumors (by biopsy) seen at Los Angeles County/University of Southern California Medical Center from 1986 to 1989. Analysis of patient age and measured tumor size in 203 fibroadenomas and 10 phyllodes tumor specimens revealed similar ranges for both tumors. The mean values were 28.5 years and 2.3 cm for fibroadenomas and 44 years and 3.8 cm for phyllodes tumors. No cases of coincident carcinoma within a fibroadenoma or of metastatic malignant phyllodes tumors were present in this review. As an alternative to excising all breast tumors, cytologically diagnosed fibroadenomas can be monitored, because they have no intrinsic premalignant potential and tend to regress with time. All breast tumors that rapidly increase in size should probably be excised at any age. (AM J OBSTET GYNECOL 1991;164:1647-51.)

Key words: Breast fibroadenoma, phyllodes tumors

Fibroadenomas are the most common benign tumors of the breast. Fine-needle aspiration cytologic testing is accurate and dependable for diagnosing fibroadenomas.[1,2] The cytologic criteria for distinguishing fibroadenomas from cancers and phyllodes tumors have been established.[3,4] Although coincident cancers do occur within fibroadenomas,[5] fibroadenomas per se are not premalignant.[6]

The clinical presentation of fibroadenoma is distinctive from cancer and phyllodes tumors. However, the definitive diagnosis must be established by fine-needle aspiration cytologic testing or histologic features on open surgical biopsy.

Must all fibroadenomas be removed by total surgical excision? Can cytologically diagnosed fibroadenomas prudently be monitored and not removed[7]? What is being achieved by the removal of all fibroadenomas? What is the cost/benefit ratio of this traditional surgical approach? What is the risk of not removing fibroadenomas? Evaluation of the Los Angeles County/University of Southern California Medical Center surgical experience for 1986 through 1989 sheds some light and suggests a conservative though provocative nonsurgical approach, to be tested by future clinical trials.

Material and methods

Computer survey of all the surgical pathology reports of breast biopsies performed in the Los Angeles County/University of Southern California Medical Center from 1986 to 1989 revealed 489 cases of fibroadenomas and 17 cases of phyllodes tumors of the breast. The age of the patient was available in all cases, but detailed specific data as to pathologic measurements of the tumors were available on only 203 fibroadenomas and 10 phyllodes tumors. Selection bias is possible because of the number of tumors that did not have precise measurements recorded in the pathology report.

Results

No coincident in situ or infiltrating cancers within fibroadenomas and no metastatic phyllodes tumors were identified. The phyllodes tumors were 3% of the total group, with a ratio of phyllodes tumors to fibroadenomas of 1:29. The patients with phyllodes tumors had a mean age of 44 years with a range of 19 to 75 years. The mean tumor size was 3.8 cm with a range of 1.5 to 8.0 cm.

The patients with fibroadenomas had a mean age of 28.5 years with a range of 12 to 65 years (Fig. 1). The mean tumor size was 2.3 cm with a range of 0.5 to 6.5 cm (Fig. 2). The 12-year-old girl had a 4 cm tumor, and the 65-year-old woman had a 0.9 cm tumor. There was a 9.5 cm juvenile giant fibroadenoma in a 16-year-old girl; it is not included in this analysis. There is a trend toward decreasing mean size with increasing age (Fig. 3).

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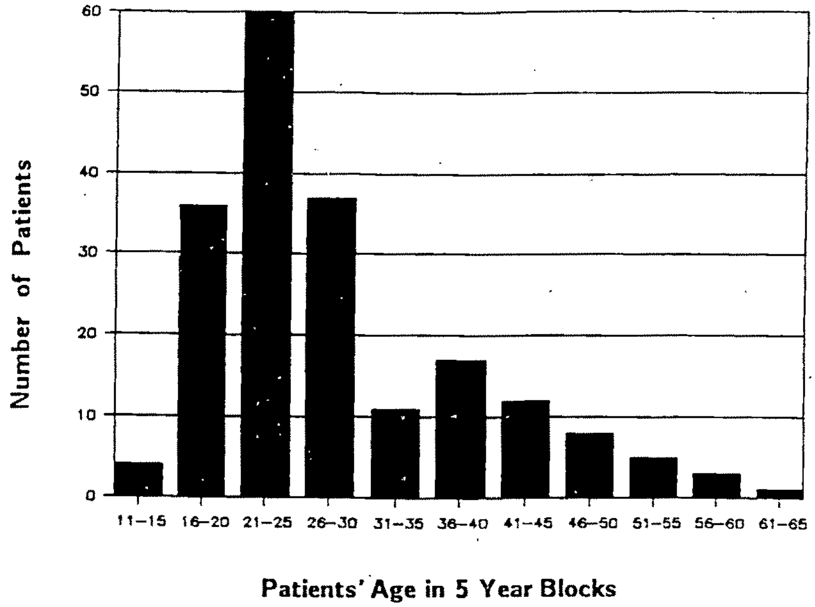

Fig. 1. Number of patients with fibroadenoma versus patient age in 5-year blocks. Total fibroadenomas, 203.

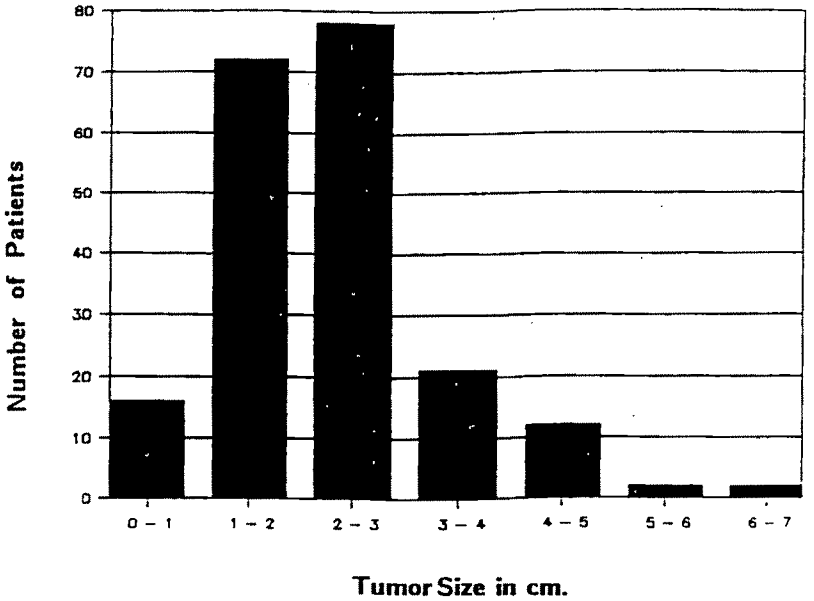

Fig. 2. Number of patients with fibroadenoma versus size of tumor by measurements recorded in pathology reports, in blocks of 1 cm. Total fibroadenomas, 203.

Comment

Treatment of breast tumors should be individualized. Options for methods of treatment should be discussed with each patient and clear, complete informed consent obtained. The choice of treatment should be a responsibly shared decision between the patient and her physician. Fear of cancer and death is a paramount anxiety in women with breast tumors, but benign lesions can be monitored with regular clinical breast examinations and mammography. Between each follow-up visit, the patient should be doing thorough, regular breast self-examinations. As with all dominant breast masses, a

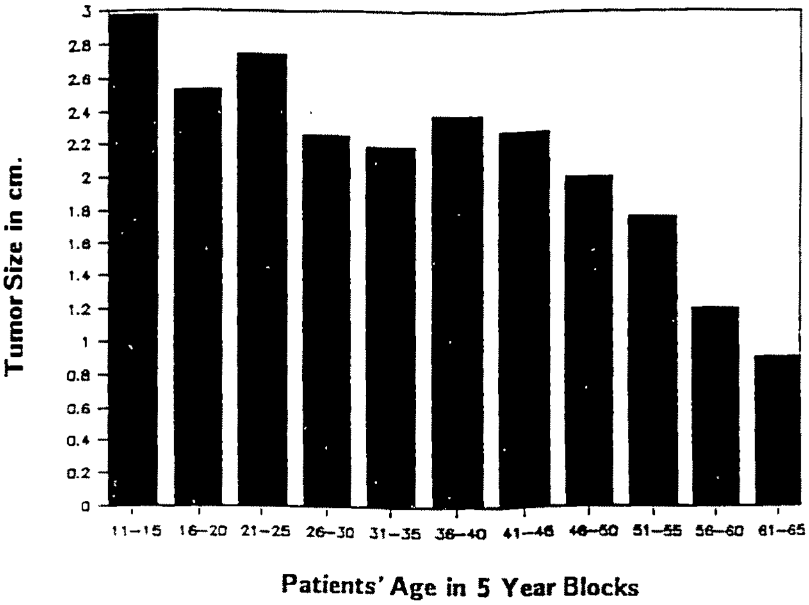

Fig. 3. Mean tumor size by measurement recorded in pathology reports of 203 fibroadenomas versus patient age in 5-year blocks.

definitive diagnosis must be established by fine-needle aspiration cytologic testing or by histologic features on open surgical biopsy.

Because of the widely overlapping ranges, the patient's age and the tumor size are not clinically reliable in differentiating fibroadenomas from phyllodes tumors. Rapid change in tumor size may be a distinctive clinical characteristic of malignant phyllodes tumors,[8,9] although fibroadenomas can increase rapidly in size during pregnancy and potentially with exogenous estrogen stimulation.

Clinically, fibroadenomas often stabilize in size at about 2 to 3 cm.[10,11] Fibroadenomas are reported to regress and resolve in significant numbers.[10,12,13] Serial mammography confirms that with aging of the patient fibroadenomas usually decrease in size, can infarct, and often calcify.[14,15] Fibroadenomas are not commonly found during autopsies or in mastectomy specimens of postmenopausal women.[12,16,17]

Phyllodes tumors have clinically been described as large (>4 cm at presentation) and rapidly growing. Phyllodes tumors are pathologically classified by established criteria as benign or malignant, although the pathologic distinction can be imprecise.[18] Although 20% of the pathologically benign tumors recur locally, they do not metastasize.[19] Twenty-six percent of the pathologically malignant tumors recur locally and 20% metastasize.[19] The usual treatment is wide excision of benign tumors and total mastectomy for malignant tumors.[20]

Future clinical research with careful follow-up after fine-needle aspiration cytologic diagnosis of fibroadenomas should be undertaken to assess the effectiveness and safety of the nonsurgical approach to fibroadenomas, to evaluate the potential decreased physical and emotional trauma to the patient, and to calculate the potential decreased health care cost.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ROGER W. HOAG, Berkeley, California. I voice a cautionary note. As we appear to be at the turning point of winning the war in the "bad baby" cases, our malpractice committees predict that the new target of the plaintiffs' attorneys will be the misdiagnosis or the delayed diagnosis of breast cancer. We are improving our skills with needle biopsy and mammography. However, if we are wrong and one of these persistent nodules turns out to be malignant, we are going to be very wrong. I have reviewed several cases in which the doctor monitored a nodule, thinking it was benign; one case was based on mammography and one case was based on a fine-needle aspiration showing a benign process. Many general surgeons who deal with breast cancer feel that if there is a mass in the breast, a report of "not cancer" on a fine-need aspiration is not sufficiently negative. We should be very careful in the next few years as these questions are debated, both in clinical meetings such as this and in the courts.

DR. CLYDE V. VON DER AHE, Los Angeles, California. I know that you have a rapid staining method and can determine, while the patient is still in the clinic, whether you are dealing with a benign or a malignant lesion. Do you recommend that the private physician practicing in an office without that ability who aspirates straw-colored clear fluid send it to a pathology laboratory to get a report stating that there are no malignant cells or simply dispose of the fluid in the sink and tell the patient it is benign? Of all the breast cysts that I have aspirated, only one cytologic result was even questionable.

DR. JACK G. HALLATT, Los Angeles, California. The value of mammography is not in question, but it is not available to some and not used by many. I tell my patients to examine their breasts whenever they bathe or shower. It takes practice to get acquainted with the normal breast tissue sufficiently to distinguish it from abnormal tissue, and I do not think it can be learned by the usually recommended once-a-month examination. Breast examination in the shower helps to overcome the emotional reluctance on the part of many women to examine their breasts. Moreover, the slipperiness of the soap film makes any abnormality stand out. Dr. Hindle, what do you feel is the value of self-examination?

DR. HINDLE (Closing). Dr. Hoag, my experience in reviewing medicolegal cases involves reviewing about one case a month and testifying in court. The best defense is to follow the guidelines, do regular examinations, and record your examinations. Absolutely every palpable mass must be diagnosed without delay. Triage of a palpable breast mass is with a needle because you immediately know, if it is a cyst, it has a 99.99% probability of being benign. If it is a solid mass, you can do fine-needle cytologic testing and obtain a specific cytologic diagnosis, for example, fibroadenoma. Without a diagnosis you have no protection. With physical examination, mammography, and fine-needle aspiration, you can specifically diagnose 90% of benign or malignant masses that are palpable in the breast. Only 10% require open biopsy. In this situation we should stop using the word *negative.* There is no such thing as a negative fine-needle aspiration or a negative mammogram. I am speaking here of diagnostic mammography used for work up in a woman with a breast problem, especially a mass. This is different from the screening mammography used for asymptomatic women.

Dr. Von Der Ahe, you can dispose of an aspirate containing clear cyst fluid provided you do two other things: make sure there is no residual mass and examine the patient to see if the cyst recurs. You need to see the patient again at 1 month and at 3 months. If there is no residual mass, if the fluid is clear or straw-colored, not bloody, and if the cyst does not recur in 3 months, the patient can return to a routine screening schedule because she is at no higher risk for breast cancer than other women her age. Bloody fluid should be sent for cytologic testing, although the incidence of carcinoma is $<1:1000$. You may have a problem if your pathologist is likely to report atypical cells. This creates a problem because you will have to do a biopsy, and that process is not cost-effective.

It is generally recognized that treatment of breast disease is a multispecialty team effort, and it is now becoming obvious that the diagnosis of breast disease is also a team effort. You must be able to talk in mutually understandable terms with your mammographer and surgeon before the biopsy and with the pathologist and cytopathologist after you do the fine-needle aspiration.

Dr. Hallatt, there are no data to show that breast self-examination done more often than once a month is any more effective. The most important thing is to do it, whatever method one uses. Women who do breast self-examination tend to have slightly earlier-stage cancer when detected. However, the average breast cancer found by self-examination is 2.5 cm, an inch, in diameter.[1] At that stage, 50% have positive nodes and 50% will die of disease.[1] I encourage every patient to do breast self-examination. When you go into court, you should have documentation in your chart that you told the patient that, not only once but every time she came back. It helps if you have shown them videos and given them handouts, but it is most important that someone has demonstrated the technique to the patient and that she has demonstrated that she has learned it.

Although breast self-examination programs will not greatly change the mortality from breast cancer, teaching breast self-examination does lead to patient awareness and willingness to participate in screening projects that are truly effective in reducing mortality. The cure rate for women with nonpalpable breast cancer <1 cm in diameter, found with screening mammography and subsequently treated, is 95% at 10 years.

When the American Cancer Societey says that one out of 10 women will get breast cancer, it does not mean you can look around a crowded room and expect that breast cancer will devlop in 10% of the women. That statistic is the cumulative life risk in the United States for a white woman who lives to be 110 years old. If you are already 50 and are free of cancer, your risk is down to <5%.[2]

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Fetal choroid plexus cysts in the second trimester of pregnancy: A cause for concern

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Controversy surrounds the issue of recommending cytogenetic studies in second-trimester fetuses with fetal choroid plexus cysts. To assist in clarifying this issue, a prospective study was designed to describe the association between fetal choroid plexus cysts and aneuploidy in a large population. During a 3-year period 7350 women at 15 to 22 weeks' gestation underwent an ultrasonographic evaluation. Fetal choroid plexus cysts were diagnosed in 71 (0.96%) of these pregnancies. Sixty-two of the 71 patients elected to undergo amniocentesis. An abnormal karyotype was identified in four (6.4%) of these fetuses. One fetus had trisomy 21, and three fetuses were diagnosed with trisomy 18. These data indicate that it is reasonable to offer genetic counseling and cytogenetic studies in those patients identified as having a fetal choroid plexus cyst. (AM J OBSTET GYNECOL 1991;164:1652-6.)

Key words: Choroid plexus cysts, aneuploidy, ultrasonography, cytogenetic studies

Recent investigations have demonstrated that various ultrasonographically visualized dysmorphic features can be used as suggestive markers in the diagnosis of the aneuploidic fetus. These features include abnormalities of the hands, feet, and limbs,[1-5] abnormalities of the face and head,[3] diaphragmatic hernia,[3] thickened nuchal skin folds,[6] congenital heart defects,[7, 8] and choroid plexus cysts.[8-10] The last of these, fetal choroid plexus cysts, has been found to be especially controversial as a morphologic variant that may be associated with aneuploidy, specifically trisomies 18 and 21.

Cysts of the choroid plexus of the lateral ventricles (Fig. 1) are relatively common in all age groups, are usually asymptomatic, and rarely produce complications.[11, 12] These cysts were initially identified in the neonate with real-time ultrasonography in 1981.[13] It was not until 1984, however, that Chudleigh et al.[14] first described this finding in the second-trimester fetus. In general, fetal choroid plexus cysts can be visualized in the late first and early second trimesters. They appear as echolucent structures, have sharply circumscribed margins, are typically <1 cm in diameter, may be either unilateral or bilateral, and usually disappear completely or significantly decrease in size by 24 to 26 weeks' gestation.[15-19] The incidence of fetal choroid plexus cysts has varied among investigators, ranging from 0.18% to 2.5%,[15, 19-22] having an average incidence of approximately 1% in all second-trimester fetuses with these cysts.[23] It is generally agreed that the majority of choroid plexus cysts are benign; however, several reports have associated cysts with chromosomal abnormalities. Both prospective and retrospective studies investigating this finding have reported on a relatively small number of cases. In addition, they reflect varied sample selection criteria, including a wide distribution of gestational ages from first- to third-trimester fetuses, and have, at times, presented anecdotal data.[8-10, 15-18, 20-23] Because of the differences among these studies, it is difficult to determine whether choroid plexus cysts do indeed have a relationship with aneuploidy. To better clarify this controversy, this report describes a prospective study, the purpose of which was to determine if an association can be demonstrated between choroid plexus cysts and chromosomal abnormalities, specifically trisomies 21, 18, and 13, in second-trimester fetuses.

Material and methods

During a 3-year period ranging from January 1987 to January 1990, 7350 women between 15 and 22 weeks' gestation underwent ultrasonographic evaluation at Cedars-Sinai Medical Center, Children's Hospital of Los Angeles, or Women's Hospital, Los Angeles County–University of Southern California Medical Center. Indications for these examinations included advanced maternal age, gestational age determinations, second-opinion ultrasonographic evaluation of a previously described abnormality, and maternal anxiety. Any women referred because of choroid plexus cysts

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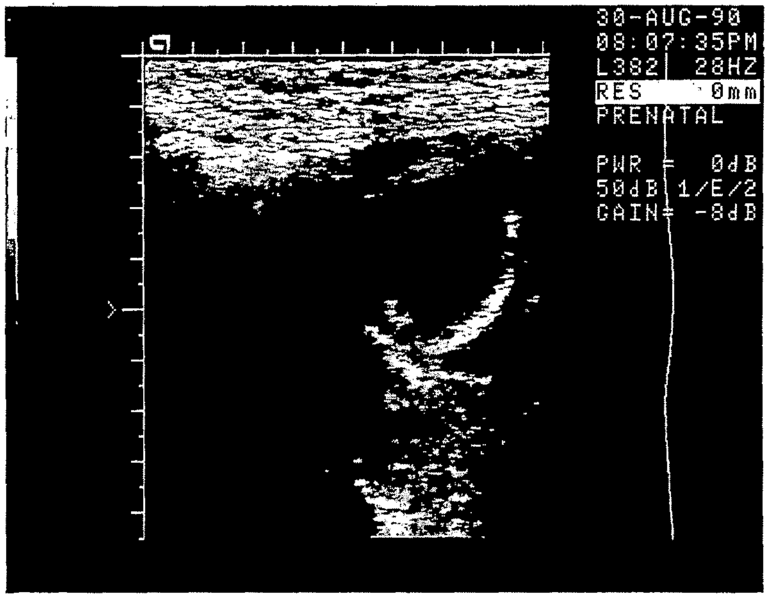

Fig. 1. Ultrasonography demonstrates presence of small, complex choroid plexus cyst.

were excluded as study patients. We performed all scans, which were done with a state-of-the-art, real-time ultrasonographic system with 3.5 to 5 MHz sector or linear transducers. Evaluations included morphometric measurement to determine gestational age, examination of the choroid plexus for the identification and measurement of a cyst, and extensive examination to detect any structural malformations. If a choroid plexus cyst was diagnosed, the patient was scheduled for a repeat ultrasonographic examination at 22 weeks. A follow-up evaluation was done at 34 weeks' gestation if the cyst was present at the time of the second examination. As a result of the diagnosis of fetal choroid plexus cyst, all women either were scheduled to undergo amniocentesis or were offered amniocentesis. Cytogenetic diagnoses were made either at the time of amniocentesis or, in those patients who declined the procedure, at the time of delivery if a dysmorphic fetus was identified.

Results

Among the 7350 women having a second-trimester ultrasonographic evaluation, fetal choroid plexus cysts were diagnosed in 71 pregnancies, an incidence of 0.96%. Fifty-eight (81.7%) of these cysts were unilateral, whereas 13 (18.3%) were bilateral. Sixty-two of the 71 patients elected to undergo amniocentesis. An abnormal karyotype was identified in four (6.4%) of these fetuses (Table I). One (1.6%) fetus had trisomy 21, and in three (4.8%) other fetuses trisomy 18 was diagnosed. Both the patient with trisomy 21 and one of the three with trisomy 18 were originally scheduled to undergo amniocentesis for previously abnormal ultrasonographic examinations.

Table I. Incidence of fetal choroid plexus cysts in relation to aneuploidy in second-trimester pregnancies

Total no. of patients scanned		7350
Fetal choroid plexus cysts identified		71 (0.96%)
Amniocenteses performed		62
No. of fetuses with aneuploidy		4 (6.4%)
Trisomy 21	1	
Trisomy 18	3	
Trisomy 13	0	

In all four aneuploidic fetuses, additional structural malformations were diagnosed by ultrasonography at the study centers. An atrioventricular canal defect was detected in the fetus with Down syndrome diagnosed. Abnormalities identified in the trisomy 18 fetuses included: (1) clenched hands, ventricular septal defect, omphalocele, and ventriculomegaly; (2) clenched hands, complex heart defect, and intrauterine growth retardation; and (3) ventricular septal defect (Table II).

Two of the women with aneuploidic fetuses subsequently terminated their pregnancies. The two remaining women with cytogenetically abnormal fetuses decided to continue their pregnancies. Fetal death occurred at 30 weeks' gestation in one of these patients, and the second was delivered at term. This neonate died eight days after birth of a congenital heart defect.

All women who continued their pregnancies were rescanned at 22 weeks' gestation. Persistent cystic

Table II. Ultrasonographic findings of aneuploidic fetuses with choroid plexus cysts

Case No.	*Chromosomal abnormality*	*Other ultrasonographic findings*
1	Trisomy 21	Atrioventricular canal defect
2	Trisomy 18	Clenched hands, ventricular septal defect, omphalocele, ventriculomegaly
3	Trisomy 18	Clenched hands, intrauterine growth retardation, complex heart defect
4	Trisomy 18	Ventricular septal defect

changes were visible in only six (5.8%) of 69 women (the 67 normal patients and the two with aneuploidic fetuses who did not terminate their pregnancies). These six cysts were completely resolved by 34 weeks' gestation. The sixth fetus died in utero, at 30 weeks' gestation.

In the 62 patients who underwent amniocentesis, all were delivered at ≥34 weeks' gestation, except for the trisomy 18 fetus that died at 30 weeks. No differences in fetal and neonatal outcome were identified between what has previously been described as complex cysts and simple cysts. Additionally, no differences in outcome were found between unilateral and bilateral cysts.

Comment

A number of studies have reported an association between fetal choroid plexus cysts and aneuploidy, particularly trisomy 18. Because of this association, it has been suggested by various researchers that cytogenetic studies be offered when these cysts are diagnosed. This recommendation is particularly inviting since several investigators[9, 10, 15, 22] have reported the presence of fetal choroid plexus cyst in aneuploidic fetuses when no other structural abnormalities were visualized ultrasonographically.

In this current study no aneuploidic fetus was noted to have a choroid plexus cyst without any other dysmorphic features identified. Of the four fetuses diagnosed with chromosomal abnormalities, however, one (25%) was detected ultrasonographically with only the diagnosis of a ventricular septal defect.

The incidence of congenital heart disease among trisomic disorders has been demonstrated to be very high. Fetuses with trisomy 21 have been reported to have a 50% incidence of cardiac anomalies. Those fetuses with trisomies 13 and 18 have a 90% and 99% incidence, respectively, with the most common of cardiac defects associated with all three disorders being ventricular septal defect.[24] A recent study has demonstrated that approximately 90% of aneuploidic fetuses were diagnosed prenatally by fetal echocardiography.[25] However, ventricular septal defects are less than optimally visualized in the second-trimester fetus,[26] especially by the less experienced ultrasonographer.

Despite the many articles advocating cytogenetic studies, the recent report by Benacerraf et al.[23] suggests that when the expected risk of amniocentesis versus the expected yield of an abnormal result is calculated, amniocentesis is not indicated when no structural malformations other than a fetal choroid plexus cyst are observed with ultrasonography. While the data in this report also demonstrate that other structural malformations were indeed seen in each of the fetuses diagnosed with aneuploidy, one patient was diagnosed with only the identification of a ventricular septal defect. However, this heart defect was sufficiently small to allow us to speculate that had optimal imaging conditions not been present, this lesion may have been overlooked by us, as well as by most ultrasonographers.

Additionally, in raising the question of the actual diagnostic value of fetal choroid plexus cysts in relation to the identification of aneuploidy, Benacerraf et al.[23] have reported a value of 30% for the sensitivity of choroid plexus cysts for detecting trisomy 18 and a positive predictive value of 0.9%. These low values appear to indicate that choroid plexus cysts are an ineffective indicator in the detection of chromosomal abnormalities. There are, however, no prospective studies that have evaluated the sensitivity, specificity, or positive and negative predictive values of the diagnosis of all malformations on ultrasonographic examination in the second trimester. Until such time that these data are available, it seems that using statistical methods for risk benefit must be limited. This type of analysis is further limited by the fact that common structural anomalies coexisting with aneuploidy, particularly congenital heart defects, may be missed.

A similar situation involves the sensitivity of ultrasonography in the diagnosis of neural tube defects. Nadel et al.[27] and Richards et al.[28] have suggested that in patients with a family history of a neural tube defect or with an elevated maternal serum α-fetoprotein level an experienced ultrasonographer is likely to rule out the vast majority of the defects. Thus, according to these authors, amniocentesis for maternal serum α-fetoprotein analysis for open neural tube defects may not be warranted. While we believe that this may have merit in some ultrasonographic hands, it may not be ready for adoption as a universal policy.

In conclusion, since the fetal head undergoes closer scrutiny by the ultrasonographer than does almost any other fetal structure, it is likely that the fetal choroid plexus cyst will be more commonly identified than other smaller defects. Once this anatomic variant is recognized, the patient should be offered, at a minimum, a second-opinion ultrasonographic examination. However, because of the data and issues presented in this report, we believe that until proper prospective studies are carried out with regard to the sensitivity and specificity of ultrasonographic diagnosis of chromosomal abnormalities in the general population and not just the "ivory tower," patients with fetal choroid plexus cysts identified should be offered genetic counseling and cytogenetic studies of the fetus by the least risky method.

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Discussion

DR. ROBERT J. LOWDEN, Seattle, Washington. Dr. Platt, you did a tremendous amount of work by reviewing 7350 ultrasonographic examinations in preparing this article. The largest similar series I found in the literature involved 6288 cases.[1] Among 7350 examinations of second-trimester patients, you found 71 fetuses with unilateral or bilateral choroid plexus cysts. Of these, 62 were subjected to amniocentesis, and four fetuses had an abnormal karyotype—one trisomy 21 and three trisomy 18. In all four an additional structural malformation was identified. In all but four of the other patients who continued their pregnancies and were scanned again at 22 weeks, the cystic changes were no longer visible. In these remaining four all cysts were gone by week 34.

Choroid plexus cysts have been identified in 1% to 3% of scans by Benacerraf et al.[2] and by Chinn et al.[3] Both felt these cysts were transient and of no clinical significance, although some were associated with trisomy 18. Chitkara et al.[1] detected choroid plexus cysts in 40 fetuses between 16 and 42 weeks' gestation. Of these, 33 resolved by 22 to 24 weeks and another four by 26 to 28 weeks. Approximately half were bilateral. One fetus with bilateral cysts and additional abnormalities had trisomy 18. Chitkara et al. strongly recommended chromosomal studies whenever associated anatomic abnormalities are detected and whenever the cysts are large, bilateral, or present after 20 to 22 weeks' gestation. Kirschbaum,[4] in his review of the article, stated that it remained to be established that choroid plexus cysts and chromosome abnormalities were more than coincidentally related.

Chinn et al.[3] reported on 13 fetuses with choroid plexus cysts among 513 women who underwent genetic amniocentesis between 16 and 24 weeks' gestation. Approximately one third of the cysts were bilateral. All 13 fetuses had normal karyotypes. Conversely, of 500 fetuses without choroid plexus cysts, five had trisomy 21 and two had trisomy 18. Benacerraf et al.[2] noted that if genetic amniocentesis was done in all second-trimester pregnancies with no ultrasonographic abnormalities other than fetal choroid plexus cysts, 478 procedures would have to be done to identify one case of trisomy 18. Clark et al.[5] in Salt Lake City found fetal choroid plexus cysts in five fetuses among patients having second-trimester ultrasonography for standard obstetric indications during a 2 year period, an incidence of 0.18%. Two cases were bilateral. No additional abnormalities were detected. All cysts had disappeared on follow-up 2 to 5 weeks later. Pregnancies continued normally and all infants were physically and neurologically normal at birth and between 4 and 24 months of age. These authors[5] concluded that in the absence of additional abnormalities and with normal sequential fetal growth, the value of routine fetal karyotyping for this indication remained undefined.

Dr. Platt, your article is informative and provocative. I agree with your point that finding a choroid plexus cyst associated with other developmental abnormalities indicates the need for a complete chromosomal study. However, on the basis of the evidence just cited, I disagree with your conclusion that the presence of choroid plexus cysts alone requires amniocentesis and further workup.

Please comment on the following. In our clinic the cost of ultrasonography, amniocentesis, and genetic workup is about $1000, on top of the cost of the usual obstetric care. Can the medical insurance industry tolerate the increased cost of care? Is there a basis for litigation if each patient does not have a workup as you advise?

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DR. FREDERICK W. HANSON, Sacramento, California. Our experience has been similar. I do not think we have had a single chromosomal abnormality in a fetus with a choroid plexus cyst without some other structural abnormality seen on ultrasonography. We have seen cysts persist as late as 28 weeks.

Dr. Platt, do you have a protocol for management of cases with choroid plexus cysts and normal amniocentesis results, normal amniotic fluid α-fetoprotein and acetylcholinesterase levels, and no other structural abnormalities seen on ultrasonography? Do you feel that there is a need for any follow-up? We now plan not to repeat the scan in patients with choroid plexus cysts <1 cm in diameter who have normal findings on amniocentesis and a level 2 ultrasonographic examination showing no other structural abnormalities.

DR. PLATT (Closing). Dr. Lowden, insurance companies pay for amniocentesis for women aged ≥35, and doing the procedure is the standard of practice for this indication. Amniocentesis for women whose fetuses have choroid plexus cysts has a higher yield of diagnoses of trisomy and should be more cost-effective. In our field we are subject to litigation by simply opening the doors of our offices. As a result, since the literature points out that some choroid plexus cysts are associated with chromosomal problems, we have the obligation to inform our patients of this relationship and offer amniocentesis. Whether the couple accepts the recommendation is entirely up to them.

Unpublished data from the California maternal serum α-fetoprotein program, which was discussed by Dr. Hanson, support my position. The recent article by Nadel et al.[1] in *The New England Journal of Medicine* suggests that when there is a high maternal serum α-fetoprotein level and a normal ultrasonographic examination, it is not cost-effective to do an amniocentesis. I believe we must realistically assess our capabilities. Ultrasonography is not a perfect science. We all will make mistakes, and even the most experienced will not be able to identify all malformations. Therefore, if we identify one of these clues to the detection of malformation, I think we need to give it careful attention.

Dr. Hanson, we first must recognize that the minute we tell the patient that her fetus has a choroid plexus cyst, her anxiety level goes through the ceiling. We have to do all we can to try to lower it by explaining that most of the cysts go away by 22 weeks. Therefore all these patients are seen by our genetic counselors, and we scan them again at 22 weeks. If the cyst persists, we offer them consultation with a pediatric neurosurgeon, who tends to lower their anxiety by telling them that these cysts are in most instances benign. We look again at 34 weeks to be sure they have not grown large enough to cause obstructive hydrocephalus. Fortunately, none of our benign choroid plexus cysts have persisted to that time.

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Outcome of very-low-birth-weight infants exposed to β-sympathomimetics in utero

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In this study we examined neonatal and early childhood outcomes after intrauterine exposure to β-sympathomimetic agents on infants with birth weights ≤1500 gm. The hospital courses and anthropomorphic, developmental, and neurologic development of 201 infants exposed to one or more β-sympathomimetic agents (isoxsuprine, 33; ritodrine, 70; terbutaline, 43; combination, 55) were analyzed and compared with those of 130 control infants of similar birth weight. One hundred and seventy-seven infants had follow-up to 1 year of age, 101 to age 3, and 58 to age 4. When treatments consisting of a single β-sympathomimetic or no treatment were compared, there were no statistically significant overall differences found in growth and development or in most of the short-term measures of infant well-being. However, significant overall differences with no evidence of confounding by time-related effects were found for the following: mortality, none greater than terbutaline; maximum positive inspiratory pressure when respiratory distress syndrome was present, none greater than terbutaline; neonatal trauma, terbutaline greater than ritodrine. Other differences were found in conjunction with evidence of time-related confounding, or within specific time intervals. It should be noted that these differences are not necessarily due to effects of the different treatments, as the data are observational. (AM J OBSTET GYNECOL 1991;164:1657-65.)

Key words: Low birth weight, β-sympathomimetics

Currently, β-sympathomimetic agents are widely used to inhibit premature labor.[1-4] In the United States ritodrine and terbutaline are currently the most popular agents. In the past isoxsuprine also was used. However, the recent withdrawal of the parenteral form of isoxsuprine from the market and its higher incidence of undesirable side effects will markedly limit its use in the future.

While there is a considerable body of literature describing side effects of the various β-sympathomimetics on the mother, data on short-term and long-term side effects on the infant are limited,[5-11] with the most information being available for ritodrine.[9, 10] Follow-up data for infants exposed to terbutaline in utero are few.[5-12]

The purpose of this study is to carefully evaluate possible adverse effects of β-sympathomimetic agents on preterm infants with birth weights of ≤1500 gm.

Material and methods

The Department of Obstetrics, Gynecology, and Reproductive Sciences has maintained a computerized perinatal data base since Nov. 1, 1975. The data base contains 300 items of information about each mother and infant delivered at our institution. It was scanned to identify all mothers delivered of infants ≤1500 gm through Dec. 31, 1983. Over this same period the Department of Pediatrics maintained a pediatric follow-up data base containing data from the immediate newborn period and from periodic follow-up visits. The two lists of cases identified in the data bases were compared, and a final list of infants with data residing in both files was generated. Only pediatric follow-up assessments through age 4 are considered in this analysis, because later assessments had small effective sample sizes (<50) that did not represent all three of the β-sympathomimetics under study.

Although the definitions of most of the items used in this analysis are apparent, several require explanation. Whenever the gestational age by history differed from the gestational age by obstetric assessment >2 weeks, the entire maternal and neonatal records were reviewed and a final gestational age was assigned by one of the authors (R.K.L.). If the patient had a prenatal visit and pelvic examination during the first 12

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Table I. Schedule of pediatric follow-up examinations

Adjusted age	*Developmental testing*	*Auditory testing*
3, 9, and 21 mo	Bayley Scales of Infant Development	Auditory brainstem responses, hearing screening
4 yr	Stanford-Binet Bayley Test of Visual Motor Integration Human Figure Drawing	Hearing screening in soundproof room

At each visit each child underwent a physical examination, including neurologic examination, and anthropometric measurements were recorded. Visual screening was done at 4 years.

Table II. Longitudinal data from the pediatric follow-up data base

- Historic data
  - Recurrent pulmonary disease
  - Other medical problems: 3 ICDA diagnoses
- Physical examination
  - Head circumference
  - Weight
  - Height
  - Abnormal physical findings
  - Hearing impairment
  - Vision impairment
  - Neurologic impairment
- If late death, age and cause of death

ICDA, International Classification of Diseases.

Table III. Preterm labor treatment data abstracted from medical record

- For each of the agents terbutaline, ritodrine, alcohol, isoxsuprine, indomethacin, and magnesium sulfate:
  - Gestational age when started
  - Route of administration
  - Was agent stopped because of complications?
  - Gestational age when stopped
  - Interval from last dose to delivery
- Corticosteroids used for lung maturation
  - Gestational age when first used
  - No. of doses given
- Complications of tocolysis
  - Pulmonary edema
  - Arrhythmias
  - Myocardial ischemia
  - Hypermagnesemia
  - Other complications: 3 ICDA codes

ICDA, International Classification of Diseases.

weeks of gestation and the gestational age agreed with the uterine size, the last menstrual period was used to determine the final gestational age. If these criteria were not met but an ultrasonographic examination had been performed during the first 20 weeks of gestation, the gestational age determined by ultrasonography was used to assign the final gestational age. If neither of these criteria was met, the gestational age by pediatric assessment[13] was used for the final gestational age. To assess clinical severity of illness in the neonatal period, each infant was given a score (illness index) ranging from 1 (no illness) to 7 (near-fatal episode[s]), as previously described.[14]

The diagnosis of the respiratory distress syndrome (RDS) (hyaline membrane disease) was based on standard clinical[15] and radiographic[16] criteria. Data for ventilatory treatment were obtained from the patients' medical records. An air leak was diagnosed if the patient had radiographic evidence of pneumothorax, pneumomediastinum, pneumopericardium, or pulmonary interstitial emphysema. Patent ductus arteriosus was diagnosed on clinical and echocardiographic criteria and was classified as absent, present but requiring no treatment, present and treated with indomethacin, present and surgically ligated, or present and treated both medically and by surgical ligation. The diagnosis of intraventricular hemorrhage was based on findings of either ultrasonographic or computerized tomographic scanning of the brain. Necrotizing enterocolitis was classified as absent, abdominal distention (suspected), intramural intestinal gas, or perforated bowel. Sepsis was diagnosed by a positive blood culture. Hypoglycemia was diagnosed by a blood glucose concentration <30 mg/dl. Trauma included the occurrence of nerve injury, fracture, laceration, or bruising. Caloric intake during the first 2 weeks of life was calculated from both enteral and parenteral feedings. The highest indirect bilirubin was the highest value recorded during the infants' hospitalization, and the highest peak inspiratory pressure was the highest ventilator setting used in the treatment of RDS.

For pediatric follow-up, each baby's age was calculated from an adjusted birth date of 40 weeks after last normal menses. The schedule of follow-up examinations is shown in Table I, and the data recorded at each visit are in Table II.

Each maternal chart was reviewed and the various data items listed in Table III abstracted. Our standard routine for the administration of β-sympathomimetics was to begin treatment with an intravenous infusion that was continued either until uterine contractions had been eliminated for 12 to 24 hours or until delivery was imminent. If and when uterine activity was controlled, oral medication was begun 1 to 2 hours before the infusion was stopped and then repeated every 1 to 4 hours. The starting intravenous infusion rates were 10 μg/min for terbutaline, 50 μg/min for ritodrine and 250 μg/min for isoxsuprine. Glucocorticoid therapy used to accelerate fetal lung maturation[17] was 12 mg betamethasone given intramuscularly and repeated once at 12 hours; two additional injection courses

Table IV. Use of tocolytic agents from 1976 through 1983 (infants with birth weight ≤1500 gm)

	Treatment											
	None		*Terbutaline only*		*Ritodrine only*		*Isoxsuprine only*		*Combination*		*Total*	
Time period	*No.*	*%**	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Overall												
No.	130	39	43	13	70	21	33	10	55	17	331	100
%	100		100		100		100		100		100	
1976-1977												
No.	33	47	0	0	0	0	25	36	12	17	70	100
%†	25		0		0		76		22		21	
1978-1979												
No.	30	44	24	35	0	0	5	7	9	13	68	100
%	23		56		0		15		16		20	
1980-1981												
No.	32	32	15	15	36	36	3	3	13	13	99	100
%	25		35		51		9		24		30	
1982-1983												
No.	35	37	4	4	34	36	0	0	21	22	94	100
%	27		9		49		0		38		28	

*Row percent, e.g., 130 = 39.3% of the row total, 331.

†Column percent, e.g., 33 = 25.4% of the column total, 130.

were given every 7 to 10 days, up to 34 weeks' gestation.

A final, complete data sheet was then generated for each case and reviewed by one of the authors (R.K.L.). All missing and obviously incorrect data were identified and corrected when possible. Statistical analysis was carried out in two stages. For the first stage, the χ^2 test (not continuity corrected) with Goodman pairwise comparisons or single-factor analyses of variance with pairwise comparisons (Bonferroni) was used; for the second stage, two-factor analyses of variance (simultaneous model/regression approach) or log-linear models for contingency tables with tests of partial association were used. Some first-stage analyses were redone with the addition of a covariate, by use of logistic regression or analysis of covariance.

Results

Three hundred thirty-one infants (263 singletons and 68 twins) delivered of 297 mothers from Jan. 1, 1976, through Dec. 31, 1983, were identified for inclusion in the study. The mothers of 130 infants (39.3%) received no tocolysis, 33 (10.0%) received only isoxsuprine, 70 (21.1%) only ritodrine, 43 (13.0%) only terbutaline; and 55 (16.6%) received either a combination of the above tocolytic agents or another agent such as ethyl alcohol, magnesium sulfate, or indomethacin. As shown in Table IV, there was a substantial change in the use of tocolytics over time. While this is explained in part by the lack of availability of ritodrine and terbutaline in the earlier years, treatment versus no treatment also varied from a no-treatment high of 47.1% in 1976 to 1977 to a low of 32.3% in 1980 to 1981 among these low-birth-weight babies ($p = 0.199$ for comparison of no-treatment rates over 2-year time periods).

The duration of tocolytic treatment averaged 2.3 days and ranged from 12 hours to 98 days. The interval between discontinuation of tocolysis and delivery was ≤24 hours in 91% of babies and <4 hours in 69%. Significant maternal side effects were uncommon but did include two cases of pulmonary edema and one of arrhythmia. Tocolysis was discontinued or the agent changed in 10% of cases to lessen side effects such as tachycardia, anxiety, and nausea.

Two hundred seventeen (66%) infants had been exposed in utero to two or more doses of corticosteroids. The mean gestational age at delivery for all infants was 28.6 weeks (range, 21 to 34 weeks); the mean birth weight was 1060 gm (range, 320 to 1495 gm). The outcome distribution for the 331 infants is shown in Fig. 1. The causes of the 15 late deaths (1 to 36 months after discharge) were sudden infant death syndrome ($n = 3$); heart disease ($n = 2$), pulmonary failure ($n = 2$), drowning ($n = 2$), acquired immunodeficiency syndrome ($n = 1$), meningitis ($n = 1$), gastroenteritis ($n = 1$), and unknown ($n = 3$). Of the 239 infants who left the hospital alive, 177 have been evaluated at least once during the first year of life, 101 were seen at 3 years of age, and 58 at age 4.

To examine various kinds of outcomes in relation to the use or nonuse of specific β-sympathomimetic agents, we excluded 55 babies who were exposed either to combinations of β-sympathomimetic agents or to other tocolytic therapies, leaving data on 276 babies for analysis. Of these, 130 had received no tocolytic agent, 43 received terbutaline, 70 ritodrine, and 33 isoxsuprine.

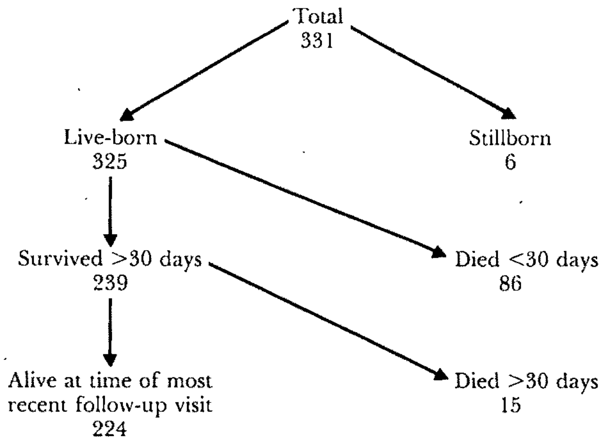

Fig. 1. Outcome of 331 infants with birth weights ≤1500 gm.

In our first-stage analyses, various maternal antepartum and intrapartum variables were compared by type of β-sympathomimetic treatment (none, isoxsuprine only, ritodrine only, terbutaline only) while time period was ignored; no significant differences were found. However, some of the neonatal and pediatric variables analyzed did show significant overall differences with respect to these treatments: trauma (Table V), mortality (Table VI), birth weight (Table VII), highest indirect bilirubin concentration (Table VIII), average caloric intake during the first week of life (Table IX), and maximal peak inspiratory pressure used during assisted ventilation for RDS (Table X). The incidence rates of RDS, patent ductus arteriosus, and intraventricular hemorrhage did not exhibit significant overall differences due to type of β-sympathomimetic treatment, and these results are not shown.

Significant pairwise comparisons between treatments (Bonferroni adjusted multiple comparisons with family significance level ≤0.05) were present for the following: trauma (Table V), terbutaline greater than ritodrine; mortality (Table VI), none greater than terbutaline; maximum peak inspiratory pressure (Table X), none greater than terbutaline; maximum indirect bilirubin (Table VIII), none greater than ritodrine, isoxsuprine greater than ritodrine and terbutaline; caloric intake (Table IX), isoxsuprine greater than none and ritodrine. A greater than B signifies that the mean or incidence for treatment A was greater than for treatment B. There were no significant pairwise comparisons for birth weight, RDS, patent ductus arteriosus, or intraventricular hemorrhage.

Although differences among treatments in overall means of gestational age were not statistically significant in this study ($p = 0.3836$), such differences could have substantial effects on some other neonatal outcomes. Therefore the nine variables just discussed were reanalyzed for overall differences among treatments with linear adjustment for gestational age. Birth weight was found to exhibit overall differences between treatments more significantly ($p = 0.0027$) with this adjustment, and a significant pairwise comparison was found: Ritodrine greater than none. For the other eight variables, adjustment produced either the same or fewer significant pairwise comparisons.

It is important to consider how many of the 66 variables analyzed might have shown significant overall differences among treatments as a result of chance alone. At the 0.05 level, six tests were found to be significant, whereas 3.3 would be expected as a result of chance alone. Five of these six were also significant at the 0.025 level, whereas only 1.65 would be expected. It is likely then that some, but not all, of these significant findings represent real differences.

Because the choice of tocolytic agents changed dramatically over time, it was important to examine the apparent differential effects of the different β-sympathomimetic agents for possible confounding by effects related to time period. To do this, subsets of the data were examined in which a single β-sympathomimetic agent had been received by at least five babies in each of at least two time periods. Within the following subsets, the indicated β-sympathomimetic agent could be compared with no treatment over the same time periods: terbutaline, 1978 to 1979 versus 1980 to 1981; ritodrine, 1980 to 1981 versus 1982 to 1983; isoxsuprine, 1976 to 1977 versus 1978 to 1979. Two kinds of time-related effects were examined: time period effects on the average over treatments (main effects of time period), and treatment-by-time-period interaction effects. If the significant time-related effects occurred with no more than chance frequency, this would suggest (but not prove) that time period reasonably could be ignored in comparing the different treatments.

Results of these analyses are summarized in the tables of means or incidence rates (Tables V to X), not including those for RDS, patent ductus arteriosus, or intraventricular hemorrhage. There were three significant tests for treatment main effect that were not invalidated by significant time-related effects: Trauma (Table V) was observed significantly more frequently in infants exposed to terbutaline than in those whose mothers were untreated ($p = 0.017$). The mortality rate was significantly higher for the untreated group as compared with infants exposed to terbutaline ($p = 0.022$) and ritodrine ($p = 0.046$) (Table VI). Finally, maximum peak inspiratory pressure when RDS is present (Table X) was significantly lower in infants exposed to terbutaline than in the untreated ($p = 0.021$).

Table V. Incidence of trauma in relation to treatment with β-sympathomimetics

Time period	*Treatment*							
	None		*Terbutaline*		*Ritodrine*		*Isoxsuprine*	
	%*	*n*†	%	*n*	%	*n*	%	*n*
Overall	16	103	32	37	9	64	14	22
1976-1977	19	21	—	0	—	0	12	16
1978-1979	13	23	29	21	—	0	25	4‡
1980-1981	15	27	46	13	14	35	—	2
1982-1983	16	32	—	3	3	29	—	0

*Incidence of trauma, when $n \geq 5$ (n is number of cases with nonmissing values on this variable).

†Significant results from first-stage analyses (p greater than 0.05): overall comparison between treatments, $p = 0.0241$; terbutaline greater than ritodrine. Significant results from subset analyses ($p < 0.05$): terbutaline versus none, 1978 to 1979 versus 1980 to 1981, treatment main effect, $p = 0.017$; terbutaline greater than none.

‡Data used in this subset analysis, as $n \geq 5$ for some variables.

Table VI. Incidence of perinatal mortality in relation to treatment with β-sympathomimetics

Time period	*Treatment*							
	None		*Terbutaline*		*Ritodrine*		*Isoxsuprine*	
	%*	*n*†	%	*n*	%	*n*	%	*n*
Overall	38	130	16	43	23	70	39	33
1976-1977	45	33	—	0	—	0	44	25
1978-1979	27	30	12	24	—	0	20	5
1980-1981	41	32	13	15	11	36	—	3
1982-1983	37	35	—	4	35	34	—	0

*Incidence of perinatal deaths, when $n \geq 5$ (n is number of cases with nonmissing values on this variable).

†Significant results from first-stage analyses ($p < 0.05$): overall comparison between treatments, $p < 0.0157$; pairwise comparisons, none greater than terbutaline. Significant results from subset analysis ($p < 0.05$): terbutaline versus none, 1978 to 1979 versus 1980 to 1981, treatment main effect, $p = 0.021$; none greater than terbutaline; ritodrine versus none, 1980 to 1981 versus 1982 to 1983, treatment main effect, $p = 0.046$; none greater than ritodrine; treatment-by-time-period interaction effect, $p = 0.043$.

Among the 48 tests of time-related effects, seven tests were significant at $p < 0.05$, whereas only 2.4 would be expected by chance. Thus most of the evidence suggesting confounding of overall treatment differences with time period effects is probably real.

Finally, when the data gathered in the longitudinal follow-up examinations were examined, no differences were found when infants of untreated mothers were compared with those exposed to the various tocolytic agents.

In summary, there were apparently real treatment differences for the short-term variables mortality and maximum peak inspiratory pressure when RDS was present. Evidence of treatment differences for trauma was somewhat inconsistent among analyses but suggested a higher incidence for terbutaline than for no treatment or ritodrine. When the various components of the trauma variable were dichotomized as present or absent, only the presence of bruising continued to show statistical significance. Conversely, there was no evidence of treatment effect or confounding for intraventricular hemorrhage. The evidence of differences among treatment groups for highest indirect bilirubin, caloric intake in the first week of life, and birth weight appears to be somewhat confounded by time period effects. Similarly, the evidence of no treatment effect for patent ductus arteriosus and RDS may be somewhat confounded.

Comment

The purpose of this study was to examine the possibility of short-term or long-term effects of various β-sympathomimetic agents on premature infants weighing ≤1500 gm. As the study design was observational, differences among treatment groups may suggest treatment effects, but effects of subject selection and uncontrolled confounding also are possible. Maternal side effects were too infrequent to allow meaningful comparison of agents on those outcomes.

The decision to use β-sympathomimetic agents for tocolysis was made on clinical grounds for individual patients by the obstetric staff; the agents were not used

Table VII. Mean birth weight in relation to treatment with β-sympathomimetics

Time period	*Treatment*							
	None		*Terbutaline*		*Ritodrine*		*Isoxsuprine*	
	*Mean**	*n†*	*Mean*	*n*	*Mean*	*n*	*Mean*	*n*
Overall	1010	130	1070	43	1102	70	1135	33
1976-1977	1010	33	—	0	—	0	1116	25
1978-1979	1069	30	1086	24	—	0	1208	5
1980-1981	931	32	1052	15	1157	36	—	3
1982-1983	1031	35	—	4	1044	34	—	0

*Mean birth weight (grams), when $n \geq 5$ (n is number of cases with nonmissing values on this variable).

†Significant results from first-stage analyses ($p < 0.05$): overall comparison between treatments, $p < 0.0360$. Significant results from subset analyses ($p < 0.05$): ritodrine versus none, 1980 to 1981 versus 1982 to 1983, treatment main effect, $p = 0.009$; ritodrine greater than none; treatment-by-time-period interaction effect, $p = 0.021$.

Table VIII. Mean highest indirect bilirubin in relation to treatment with β-sympathomimetics

Time period	*Treatment*							
	None		*Terbutaline*		*Ritodrine*		*Isoxsuprine*	
	*Mean**	*n†*	*Mean*	*n*	*Mean*	*n*	*Mean*	*n*
Overall	7.63	90	7.09	39	6.69	61	8.76	22
1976-1977	8.61	20	—	0	—	0	9.19	16
1978-1979	8.33	23	6.92	23	—	0	8.15	4‡
1980-1981	6.84	21	7.05	13	6.96	35	—	2
1982-1983	6.89	26	—	3	6.32	26	—	0

*Mean indirect bilirubin (milligrams per deciliter), when $n \geq 5$ (n is number of cases with nonmissing values on this variable).

†Significant results from first-stage analyses ($p < 0.05$): overall comparison between treatments, $p < 0.0006$: pairwise comparisons, none greater than ritodrine, isoxsuprine greater than ritodrine and terbutaline. Significant results from subset analyses ($p < 0.05$): terbutaline versus none, 1978 to 1979 versus 1980 to 1981, treatment-by-time-period interaction effect, $p = 0.036$; 1978 to 1979 greater than 1980 to 1981.

‡Data used in this subset analysis, as $n \geq 5$ for some variables.

in these patients under a protocol for a prospective clinical trial. Thus the evaluation of the outcome of the infants was based on a retrospective review of our neonatal and follow-up data.

It may be asked why the different β-sympathomimetic agents were considered separately in these analyses, rather than being compared collectively with no treatment. Because different degrees of β-adrenergic receptor selectivity have been demonstrated for the agents, we thought it prudent to consider possible clinical effects of such differences.

Of the major neonatal outcome variables, only mortality showed a significant difference, with the group receiving terbutaline or ritodrine having a lower mortality rate than the untreated group. The cause for this lessened mortality is not apparent but may be related to the higher birth weights in the β-sympathomimetic group, although the average difference in birth weight between the two groups was small (83 gm). Because this was not a controlled trial, the differences in mortality rates should be interpreted with caution.

Fifteen deaths occurred after the neonatal period; there is no evidence to indicate that these were caused by the maternal β-sympathomimetic treatment. The occurrences of three cases of sudden infant death syndrome in this group is similar to the incidence that has previously been reported among infants of low birth weight.[18]

β-Sympathomimetic agents are known to have marked effects on the fetal lung in experimental animals. These effects include release of pulmonary surfactant,[19-21] decreased lung liquid production,[21, 22] resorption of alveolar fluid,[22] and a decrease in lung water content[19, 23]; all of these actions might be expected to decrease RDS. Conversely, treatment with β-sympathomimetic agents also has been reported to deplete surfactant stores,[24] an effect that might increase the incidence of RDS. In any case we found no difference in incidence of RDS between the infants receiving any one of the β-sympathomimetics and those receiving none. There are at least two possible explanations: (1) The effects of β-sympathomimetics are less pronounced in human fetuses than in laboratory animals; (2) an effect similar to that of β-sympathomimetics may

Table IX. Mean average caloric intake in first week of life in relation to treatment with β-sympathomimetics

Time period	*Treatment*							
	None		*Terbutaline*		*Ritodrine*		*Isoxsuprine*	
	*Mean**	*n†*	*mean*	*n*	*Mean*	*n*	*Mean*	*n*
Overall	48.2	87	52.7	37	49.3	61	60.5	19
1976-1977	51.2	20	—	0	—	0	60.5	13
1978-1979	50.2	23	57.9	21	—	0	56.5	4‡
1980-1981	47.8	20	47.9	13	52.9	35	—	2
1982-1983	44.0	24	—	3	44.3	26	—	—

*Mean caloric intake (calories per kilogram per day during first week of life), when $n \geq 5$ (n is number of cases with nonmissing values on this variable).

†Significant results from first-stage analyses ($p < 0.05$): overall comparison between treatments, $p < 0.0109$; pairwise comparisons, isoxsuprine greater than none and ritodrine. Significant results from subset analyses ($p < 0.05$): ritodrine versus none, 1980 to 1981 versus 1982 to 1983; time period main effect, $p = 0.020$; 1980 to 1981 greater than 1982 to 1983.

‡Data used in this subset analysis, as $n \geq 5$ for some variables.

Table X. Mean of maximum peak inspiratory pressure when RDS was present in relation to treatment with β-sympathomimetics

Time period	*Treatment*							
	None		*Terbutaline*		*Ritodrine*		*Isoxsuprine*	
	*Mean**	*n†*	*Mean*	*n*	*Mean*	*n*	*Mean*	*n*
Overall	28.0	57	22.2	19	24.8	37	29.6	9
1976-1977	29.5	10	—	0	—	0	30.8	8
1978-1979	23.7	12	22.1	7	—	0	—	0
1980-1981	28.3	14	20.5	10	21.1	19	—	1
1982-1983	29.5	21	—	2	28.6	18	—	0

*Mean maximum peak inspiratory pressure requirement (cm H_2O), when $n \geq 5$ (n is number of cases with nonmissing values on this variable).

†Significant results from first-stage analyses ($p < 0.05$): overall comparison between treatments, $p < 0.0194$; pairwise comparisons, none greater than terbutaline. Significant results from subset analysis ($p < 0.05$): terbutaline versus none, 1978 to 1979 versus 1980 to 1981, treatment main effect, $p = 0.021$; none greater than terbutaline.

occur in the process of labor. Of interest was the finding that maximal peak inspiratory pressure was significantly lower in infants with RDS in the terbutaline group than in the no-treatment group, suggesting that the lung disease was milder. It was also lower in the ritodrine-treated group but not significantly so.

The lowest caloric intake during the first week of life was in the untreated group. This may have been because they were more unstable and less tolerant of fluids and enteric feedings. Although there were statistically significant differences in the maximal concentration of indirect bilirubin, the actual differences were small and were not clinically important.

Of the 37 cases found to have some form of trauma, all but six had bruising. These six consisted of minor lacerations ($n = 1$), forceps marks ($n = 4$), and abrasions ($n = 1$). There were no cases of nerve injury or fracture. We see no clear explanation of the apparent excess of these minor traumas in infants exposed to terbutaline.

Samples sizes for comparisons between types of β-sympathomimetic treatments on long-term follow-up assessment variables (Tables I and II) were modest at best. Anthropometric measurements (head circumference, height, weight) were made on 100 to 120 subjects at age 3 and 21 months but only on smaller numbers at other ages. Intelligence quotient was measured in 130 subjects at age 1 year, in 117 at age 2, and in smaller numbers of older children. In the comparisons involving sample sizes of ≥100, about 50 subjects had received no β-sympathomimetic agents, 25 to 35 had received ritodrine, 20 to 30 had received terbutaline, and 10 had received isoxsuprine. Therefore only rather gross differences between treatments in long-term outcome could have been detected as statistically significant.

Our experience suggests that the use of β-sympathomimetic agents for tocolysis does not have any substantial, statistically significant adverse effects on the neonatal course of infants whose birth weights were ≤1500 gm. While statistically significant adverse effects were not found in the longer-term outcomes, these comparisons are very insensitive, particularly after the first year of life, because of marked reductions in the number of subjects for whom results were obtained. The lower mortality rate in the β-sympathomimetic group should not be directly attributed to the use of β-sympathomimetic agents.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. DURLIN HICKOCK, Seattle, Washington. Dr. Laros has presented an important article describing both the short- and long-term effects of β-sympathomimetics on the infant exposed in utero. In this cohort of infants born at the University of California, San Francisco, 210 infants exposed to one or more β-sympathomimetic drugs were compared with 130 infants of similar birth weights whose mothers did not receive β-sympathomimetic drugs. Few differences were found in the long-term variables examined. The only consistent short-term differences between the treated and untreated groups appeared to be a reduction of perinatal mortality in infants whose mothers were treated with ritodrine or terbutaline and a lower peak inspiratory pressure when RDS was present.

In metaanalyses of placebo-controlled, randomized trials, the β-sympathomimetics are highly effective for prolonging delivery >48 hours, although to a lesser extent preventing birth before the thirty-seventh completed week. No significant effect on reduction of perinatal death has been demonstrated to date. Furthermore, from experimental studies there appears to be no influence on the risk of RDS.[1]

All of the β-sympathomimetics cross the placenta, although the ratio of fetal-to-maternal serum levels varies with the different drugs. Fetal effects, for the most part, parallel closely those observed in the mother. After β-sympathomimetic infusion, predictable increases in glucose, lactate, and insulin occur, with a return to baseline levels within 72 hours, with the exception of lactate, which remains elevated. Neonatal hypoglycemia, hypocalcemia, ileus, and hypotension have been reported to be more common in infants whose mothers were treated with isoxsuprine and who were delivered within 48 hours of treatment.[2]

Much less is known regarding potential long-term effects on the fetus from maternal β-sympathomimetic therapy. Only 18 placebo-controlled trials involving <500 women allocated to β-sympathomimetic treatment have been reported, and few of these studies involved infant follow-up beyond the initial hospital stay. Reports to date, however, have been reassuring with lack of significant long-term effects reported by Polowczyk et al.[3] in 20 children and by Dr. Laros in the current study.

Dr. Laros is to be congratulated in presenting an important study. All too often the obstetric literature concentrates on studies of clinical efficacy of a therapy or intervention for pregnancy disorders and loses sight of the important issue of clinical safety. The effectiveness of treatment is often much easier to measure, less costly to study, and more exciting in its presentation. Clinical safety, nonetheless, remains as important as clinical efficacy in the evaluation of new treatments and technologies. We have already experienced the unfortunate example of the excess risk of vaginal adenocarcinoma caused by in utero exposure of diethylstilbestrol.

Any comments I have to Dr. Laros should be considered as potential limitations rather than criticisms. In a nonexperimental study such as this, many of the determinants of the outcomes of interest cannot be adequately controlled for, thus limiting the generalizability of the results. The lack of serious adverse effects in this study, however, remains an important finding. I have several questions for Dr. Laros, mainly centering around the issue of those who were untreated: Were these patients too advanced in labor to be treated? What proportion of the nontreated group were deemed not to be candidates for therapy because of rupture of membranes?

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Dr. Robert C. Goodlin, Denver, Colorado. This is a much-needed study. I have just two questions. Have you looked at the incidence of fetal growth retardation among these prematurely born babies? It might make a great difference in outcome. In Europe, terbutaline is considered effective therapy for prevention of hyaline membrane disease,[1] just as we think steroids are effective. Do you agree that it is?

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Dr. Richard N. Bolton, Portland, Oregon. "Bruising," like beauty, is in the eye of the beholder. There are three groups, each divided into 2 years—isoxsuprine, terbutaline, and ritodrine. Could there have been an observer during the terbutaline years, not present during the isoxsuprine or ritodrine years, who, because of personal sensitivity, saw and recorded more "bruising"?

Dr. Thomas Garite, Orange, California. The safety of therapy is a very important issue. Long-term follow-up is obviously missing in these patients. In comparing a group that received no tocolytic agents with a group who received these agents, the difference in outcome seen might be explained either by differences in the agents or by other differences between the two groups. When the patient is not a candidate for tocolysis or when the drug is contraindicated, she is more likely to have a problem that predisposes the baby to birth asphyxia and neonatal complications. Examples are chorioamnionitis, abruptio placentae, and premature rupture of membranes, the latter predisposing to umbilical cord compression. Dr. Laros, was any effort made to see whether the condition at birth might have explained any differences in perinatal mortality, Apgar scores, umbilical cord blood gases, or other outcome?

Dr. Laros (Closing). Dr. Hickok, you are absolutely correct. There was a difference in the *none* group. The vast majority of patients in this group, on the order of 80%, were not considered candidates for tocolysis because of relatively far advanced cervical dilation when they were first seen in our unit. Approximately 10% of the *none* group had premature rupture of membranes, and in about 5% or 10%, lack of treatment was apparently just a matter of choice by the attending physician.

Dr. Goodlin and Dr. Hickok, there are two clear studies, one from Germany and one recent one from the United States, pointing out that, despite the wide use of β-sympathomimetics and other tocolytic agents, there has been no decrease in the incidence of preterm delivery. We have to continue to ask ourselves exactly what are we doing.

Dr. Garite, we did look quite carefully at confounding factors that might have an effect on outcome. There was no difference in the incidence of chorioamnionitis in the four treatment groups. We did have cord blood gases and Apgar scores on virtually all of these babies, and there were no differences among the groups. One is always in the position of back filling when one tries to convince the reader that the various groups in an observation study are equal. We are as comfortable as we can be from an epidemiologic viewpoint that these were comparable groups and that the differences observed or not observed are due to treatment and not to ascertainment bias.

Dr. Goodlin, the incidence of intrauterine growth retardation was not different in the various groups.

Dr. Bolton, I also have been suspicious that the eye of the beholder is an important issue. Unfortunately, we could not explain away the *trauma* issue because our second level of analysis took into account time, which should control for different observers.

Ten-year follow-up of patients with secondary amenorrhea and normal prolactin

Val Davajan, MD,[a] Oscar Kletzky, MD,[b] Michael Vermesh, MD,[a] and Debra J. Anderson, MD[a]
Los Angeles and Torrance, California

A group of 46 patients with secondary amenorrhea without galactorrhea or hyperprolactinemia were studied retrospectively after being clinically categorized into four groups with the use of progesterone-induced uterine bleeding and measurement of serum gonadotropins and prolactin levels. The ability to have regular spontaneous menstrual cycles and to conceive was assessed after a follow-up period of 10 years. Patients who had been classified as having hypothalamic pituitary "failure" (hypoestrogenic amenorrhea) with low levels of circulating estradiol had a greater rate of recovery of spontaneous ovulation and menses when compared with patients who had been classified as having only hypothalamic pituitary dysfunction (euestrogenic amenorrhea). The patients with diagnosis of hyperandrogenic chronic anovulation or polycystic ovary syndrome generally required clomiphene citrate for induction of ovulation and almost all the patients with premature ovarian failure (hypergonadotropic amenorrhea) remained estrogen-deficient and unable to ovulate. Hyperprolactinemia or an identifiable pituitary adenoma has not developed in any of the patients to date. (AM J OBSTET GYNECOL 1991;164:1666-72.)

Key words: Secondary amenorrhea, hyperprolactinemia, euestrogenic secondary amenorrhea, hypoestrogenic secondary amenorrhea, hypergonadotropic secondary amenorrhea

Patients with secondary amenorrhea and normal serum prolactin (PRL) levels can be clinically categorized into four groups with the use of progesterone or progestin-induced uterine bleeding and measurement of a single serum gonadotropin level.[1] Those with uterine bleeding after administration of progesterone were reported to have serum estradiol levels >40 pg/ml (euestrogenic amenorrhea) and were further subdivided into two groups on the basis of serum luteinizing hormone (LH) levels. Elevated serum LH levels ≥25 mIU/ml or an LH/follicle-stimulating hormone (FSH) ratio greater than three are seen in patients with polycystic ovary syndrome, also referred to as hyperandrogenic chronic anovulation.[2] Hypothalamic dysfunction was diagnosed in patients with normal serum LH levels. Patients with no uterine bleeding to progesterone challenge (<40 pg/ml of estradiol, hypoestrogenic amenorrhea) were further subdivided into group of those with hypothalamic pituitary failure if the serum FSH level was low or low-normal and those with premature ovarian failure (hypergonadotropic hypogonadism) if the serum FSH level was noted to be elevated into the menopausal range.

This 10-year retrospective study analyzed the reproductive changes that occurred in 46 women with secondary amenorrhea and normal serum (PRL) levels, specifically to the prevalence of establishment of spontaneous normal menses and ability to conceive.

Material and methods

A group of 46 patients with secondary amenorrhea without galactorrhea or hyperprolactinemia were studied retrospectively. At the initial visit between 1974 and 1977, these patients had undergone 6 to 48 months of amenorrhea. Their ages ranged between 18 to 34 years (Table I). Eighteen (39%) patients had normal menses and 28 (61%) had oligomenorrhea before onset of amenorrhea. In addition to those with hyperprolactinemia, patients with hypothyroidism or diabetes mellitus were excluded from the study. Each patient on initial presentation had a complete medical history and physical examination that included a pelvic examination, a thorough breast examination to exclude galactorrhea, and appropriate routine laboratory tests. The possible confounding factors for each group of patients are listed in Table II. An intramuscular injection of 100 mg of progesterone in oil or medroxyprogesterone acetate (Provera) 30 mg orally for 3 days was given to all 46 patients. Those who responded with uterine bleeding 2 to 14 days after the administration of progesterone (positive response) had a serum LH level deter-

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Table I. Secondary amenorrhea patient data ($N = 46$)

	PCO ($n = 13$)		*HD* ($n = 17$)		*HPF* ($n = 9$)		*POF* ($n = 7$)	
	No.	*Range*	*No.*	*Range*	*No.*	*Range*	*No.*	*Range*
Age (yr)	24.1	18-32	27.7	22-34	23.6	19-28	27.7	22-33
Age at menarche (yr)	11.7	9-16	12.3	10-18	13.2	11-16	14.3	11-16
Nulliparous	12	—	16	—	8	—	7	—
Paras	—	—	—	—	1	—	—	—
No. of abortions	1	—	1	—	—	—	—	—
Amenorrhea	9 = 48 mo	—	7 = 16 mo	—	9 = 16 mo	—	6 = 14 mo	—

PCO, Polycystic ovary syndrome (hyperandrogenic chronic anovulation); *HD*, hypothalamic dysfunction (euestrogenic amenorrhea); *HPF*, hypothalamic pituitary failure (hypoestrogenic amenorrhea); *POF*, primary ovarian failure (hypergonadotropic amenorrhea).

Table II. Secondary amenorrhea confounding factors (N = 46)

	PCO	*HD*	*HPF*	*POF*	*No.*
Unknown	2	6	3	7	18
Weight loss	—	3	4	—	7
Anorexia nervosa	—	—	1	—	1
Excessive exercise	—	2	1	—	3
Medications	—	6	—	—	6
Weight gain	11	—	—	—	11
TOTAL	13	17	9	7	

PCO, Polycystic ovary syndrome; *HD*, hypothalamic dysfunction; *HPF*, hypothalamic pituitary failure; *POF*, primary ovarian failure.

mined. If hirsutism, obesity, or acne was also present in this group of patients serum FSH, dehydroepiandrosterone (DHEA) sulfate and total serum testosterone levels were also measured. The initial four patients in the series were seen before serum DHEA-sulfate levels were available and therefore had 24-hour urinary 17-ketosteroid values determined. Routine diagnostic laparoscopy or ultrasonography were not used in this study to confirm the diagnosis of polycystic ovary syndrome or hyperandrogenic chronic anovulation.

Patients who did not respond with uterine bleeding after administration of progesterone (negative response) had serum FSH level measured. Only hypoestrogenic patients with low or normal serum FSH levels (<10 μIU/ml) had hypocycloidal polytomograms ($n = 5$) or computed tomography (CT) scans ($n = 4$) of the sella turcica. These initial studies were done before use of magnetic resonance imaging.

Serum LH, FSH, TSH, PRL, testosterone, and DHEA- sulfate were determined by previously reported radioimmunoassay methods.[3] Significance of differences between initial hormone values and those obtained 10 years later in the same patients were obtained with the use of the paired Student *t* test. Results are reported as mean ± SEM.

Results

There were 30 patients with a positive uterine bleeding response to progesterone and 16 with a negative response. Any amount of bleeding was considered positive. Of the 30 patients with positive response, the diagnosis polycystic ovary syndrome (hyperandrogenic chronic anovulation) was made in 13 patients because they had either an elevated serum LH level of ≥25 mIU/ml ($n = 8$)[4] or an LH/FSH ratio ≥3 ($n = 5$). In addition, all 13 patients had one or more physical signs of hyperandrogenism such as obesity, hirsutism, and acne. In this group the mean serum testosterone level was 104.5 ± 4.85 ng/dl (normal, 20 to 85 ng/dl), mean DHEA sulfate was 3.26 ± 0.19 (normal, 0.5 to 2.8 μg/ml) (Fig. 1). Intermittently all 13 patients were treated with cyclic oral contraceptives; only one patient was treated with dexamethasone 0.25 mg daily orally at bedtime, and four with significant acne were treated with spironolactone (100 mg orally daily). Nine of these patients desired pregnancy and were treated with clomiphene citrate. None of the 13 patients spontaneously began to have regular menstrual cycles in the 10 years of follow-up, but after periodic discontinuation of oral contraceptives (from 1 to 6 months) all continued to have withdrawal uterine bleeding in response to progesterone administration. There was either no progression in the hirsutism ($n = 5$) or there was improvement in both the hirsutism ($n = 8$) and acne ($n = 6$) while being treated. After 10 years, the mean serum testosterone level of this group decreased to 70.2 ± 5.1 ng/dl ($p \leq 0.005$) and the DHEA sulfate level decreased to 2.3 ± 0.14 μg/ml ($p \leq 0.005$) (Fig. 1). During the same time period nine patients increased in body weight and as a group body weight increased from 156 ± 5.0 pounds (70.5 kg) to 177 ± 8.2 pounds (80.5 kg) ($p < 0.05$). Four of the nine patients who desired conception achieved at least one pregnancy with clomiphene citrate.

The remaining 17 of the 30 patients with positive response had a normal initial serum LH level of 10.5 ± 0.75 (normal values, 4 to 20 mIU/ml) and 10 years later it was 12.4 ± 1.0 mIU/ml (Fig. 2). Euestro-

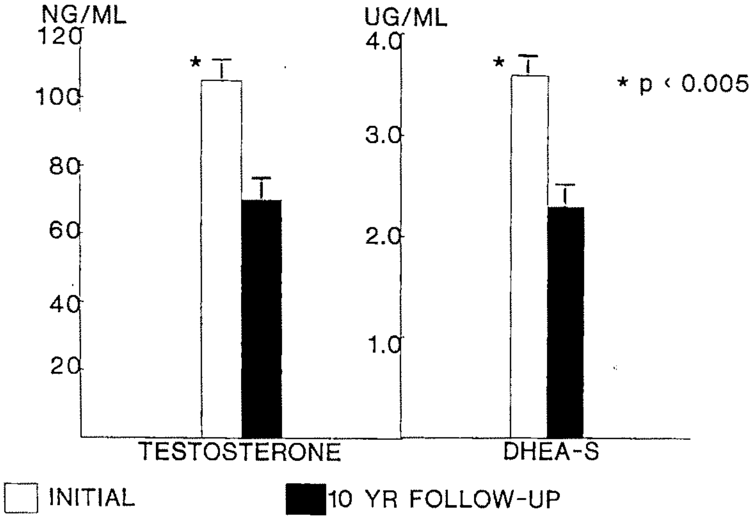

Fig. 1. Secondary amenorrhea: polycystic ovary syndrome serum androgens. Results of serum androgens in initial and 10-year follow-up in the patient with polycystic ovary syndrome (hyperandrogenic chronic anovulation).

genic secondary amenorrhea as a result of hypothalamic dysfunction was diagnosed in these patients. Before onset of amenorrhea three had lost at least 15% of their body weight, two reported to be engaged in excessive physical exercise.[5-7] In six the amenorrhea followed the discontinuation of oral contraceptives.[8] All 17 patients were treated with oral progestin (5 to 10 mg daily) for 10 to 12 days every 60 days and clomiphene citrate was used to induce ovulation in the 12 who wished to conceive. For patients who desired contraception the use of vaginal diaphragm or condoms was recommended.

After 10-year follow-up, all 17 patients discontinued all medications for 1 to 6 months before reevaluation. Serum PRL values remained in the normal range (6 to 20 ng/ml) (Fig. 3) in all but one patient who was taking a psychotrophic drug. The initial mean value was 9.5 ± 0.84 and 10 years later it was 12.8 ± 1.41 ng/ml (± SEM, not significant). During follow-up, three patients resumed spontaneous ovulatory cycles as demonstrated by biphasic basal body temperature and serum progesterone level of ≥8 ng/ml. Oligomenorrhea developed in four patients and 10 continued to have secondary amenorrhea with positive response to progesterone. Six of the 12 patients treated with clomiphene citrate conceived and had at least one successful term delivery. An additional patient conceived spontaneously.

Of the 16 patients with negative response after progesterone administration, nine (aged 19 to 28 years) had a mean serum FSH level of 3.8 ± 0.62 mIU/ml (normal, 2.8 to 9 mIU/ml) and seven (aged 22 to 33 years) had a level of 99.9 ± 8.2 mIU/ml. Hypoestrogenic amenorrhea or hypothalamic pituitary failure was diagnosed in patients with low or low-normal serum FSH levels. Six of nine patients had regular cycles before the sudden onset of amenorrhea. All six had either ≥20% loss of body weight ($n = 5$) or performed excessive physical activity ($n = 1$). The remaining three patients had no known confounding factor. Hypocycloidal polytomography or computed tomography scan studies of the sella turcica did not show any evidence of pituitary adenoma in patients with hypothalamic pituitary failure. Serum cortisol, growth hormone, and PRL response was normal after insulin-induced hypoglycemia,[9] and stimulation with gonadotropin releasing-hormone induced a normal serum LH and FSH response in all patients.[4] These patients were initially treated with replacement oral estrogen/progestin therapy (conjugated estrogen, 0.625 mg orally daily for 25 days and medroxyprogesterone acetate, 5 to 10 mg daily for 12 days). The duration of treatment was variable. During follow-up, six patients recovered spontaneous ovulatory menstrual cycles. Mean serum FSH levels were normal initially (3.8 ± 0.62) and after 10 year follow-up (5.5 ± 0.42 mIU/ml). Mean serum PRL values (10.4 ± 1.17) did not change significantly after 10 years (15.8 ± 5.2 ng/ml) (Fig. 3). Six of these nine patients recovered normal spontaneous menstrual cycles. Three patients remained amenorrheic; however, two of these patients were euestrogenic and began to bleed in response to progesterone and therefore hypothalamic dysfunction was diagnosed. Only one patient remained with the diagnosis of hypothalamic pituitary failure and subsequently had normal results of a magnetic resonance imaging study of the pituitary. All six patients who wanted to conceive did so either spontaneously or after treatment with clomiphene citrate.

Premature ovarian failure or hypergonadotropic

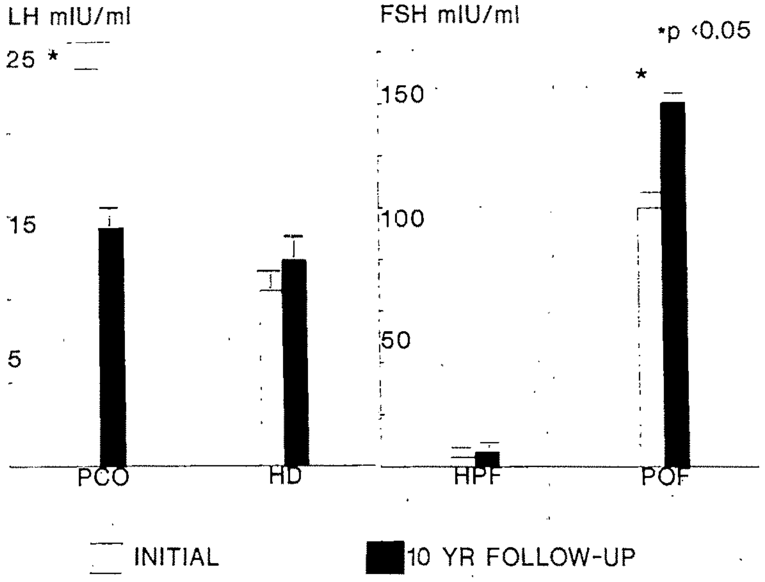

Fig. 2. Secondary amenorrhea: gonadotropins. Results of pituitary gonadotropins in the four groups (initial and 10-year follow-up).

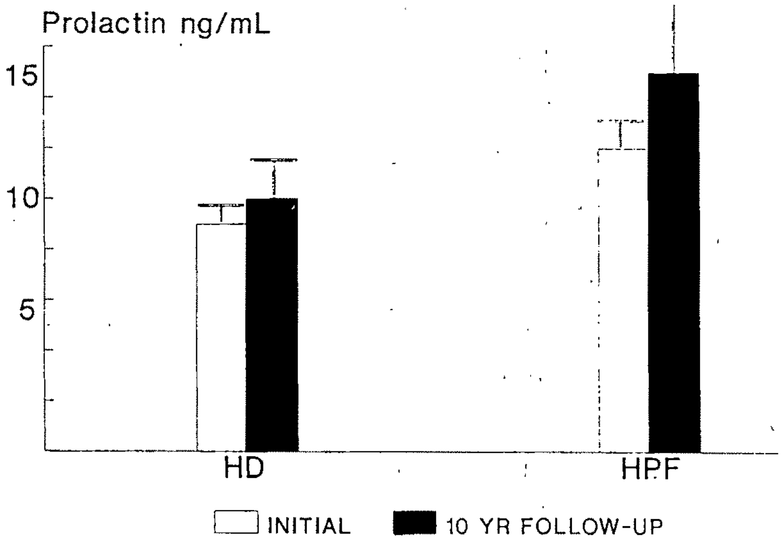

Fig. 3. Secondary amenorrhea: PRL. Serum PRL levels (initial and 10-year follow-up) in patients with hypothalamic dysfunction (euestrogenic amenorrhea) and hypothalamic failure (hypoestrogenic amenorrhea).

amenorrhea was diagnosed in the remaining seven patients with a negative response to progesterone. Five women had an acute onset of amenorrhea. Mean serum FSH level initially was 99.9 ± 8.2 and 10 years later it was 140.4 ± 9.2 mIU/ml ($p \leq 0.05$) (Fig. 2). Antithyroid and antimicrosomal antibody test results were negative in all patients.[10] The three patients younger than 25 years had normal 46,XX karyotype. In the 10-year follow-up two patients had spontaneous but sporadic uterine bleeding episodes. One of the two had a 6-month interval of monthly menstrual bleeding during the sixth year of follow-up. Serum FSH value was 18 mIU/ml during the fourth month of this "recovery" period. All patients had been treated with estrogen-progestin replacement therapy and discontinued all medications for at least 1 month before reevaluation. None of the seven patients conceived although four were treated with high doses of oral estrogen in an effort to trigger ovulation by the "rebound" phenomena.[11]

Comment

The results of this study indicate that patients with hypoestrogenic secondary amenorrhea as a result of low gonadotropin values as a consequence of weight loss and strenuous exercise have a good prognosis as to recovery of normal menstrual cycles (66%). In addition, only three of 17 patients with euestrogenic amenorrhea as a result of hypothalamic dysfunction were having normal menstrual cycles at the end of 10 years (17.6%). Amenorrhea developed in two of these three patients after discontinuation of the oral contraceptives and one was a result of weight loss. In contrast, no patient with hyperandrogenic amenorrhea, polycystic ovary syndrome, or premature ovarian failure recovered spontaneous ovulatory menstrual cycles even after 10 years of follow-up. It has been suggested that patients with hypothalamic euestrogenic amenorrhea have a hypothalamic derangement, whereas patients with hypoestrogenic amenorrhea have either a more profound gonadotropin-releasing hormone deficiency or an intrinsic pituitary alteration.[12] It was therefore assumed that these patients would not resume a spontaneous menstrual cycle. The results of this study did not bear this out. As stated earlier, only three of 17 (18%) patients with euestrogenic amenorrhea (hypothalamic dysfunction) were having spontaneous normal menstrual cycle as compared with six of nine patients with hypoestrogenic amenorrhea (hypothalamic pituitary failure) (67%). Analysis of these patients' abilities to conceive revealed that 17 of 27 (63%) patients with the collective diagnoses of hyperandrogenic, euestrogenic, and hypoestrogenic amenorrhea who wished to conceive were able to do so either spontaneously or after treatment with clomiphene citrate. In contrast, no patient with premature ovarian failure was able to conceive. In this study human menopausal gonadotropin therapy was not used to induce ovulation in patients who did not conceive with the use of clomiphene citrate. During the majority of the time that this study was undertaken, human menopausal gonadotropin therapy was not being used to induce ovulation except in patients with hypoestrogenic amenorrhea. Because all the patients in this group who desired to conceive ovulated spontaneously or responded to clomiphene, human menopausal gonadotropin therapy was not used in this study. This is in marked contrast with what is offered to patients today. Even women who ovulate spontaneously and do not conceive are treated with human menopausal gonadotropin.

In patients with hyperandrogenic amenorrhea, the hirsutism was either improved with regard to its courseness and retarded with regard to its speed of regrowth ($n = 8$) or remained unchanged ($n = 5$). None of the patients had worsening of hirsutism. All patients reported overall improvement in skin condition. All these patients showed improvement in androgen levels on therapy. It should be noted that the hirsutism was not completely eradicated and therefore appears to be permanent once it is present. The most chronic complaint encountered in following up the 13 patients with hyperandrogenic amenorrhea was the difficulty in weight control. Although all four groups of patients gained weight in the 10-year follow-up, the patients with hyperandrogenic amenorrhea were heavy to begin with and continued to gain weight.

It should be noted that hyperprolactinemia did not develop in any of the patients with hypothalamic dysfunction or hypothalamic pituitary failure in the 10-year follow-up. In conclusion, this longitudinal study, although relatively small in number of patients, provides guidelines for counseling patients with amenorrhea as to prognosis for spontaneous recovery of menses and chance of conception.

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Discussion

DR. TAWFIK H. RIZKALLAH, Phoenix, Arizona. Dr. Davajan classified 46 normoprolactinemic patients with secondary amenorrhea into four categories. This classification was dependent on the patient's response to administered progesterone or progestational agent, androgenic state, serum estradiol, and FSH levels. With this in mind, Dr. Davajan retrospectively analyzed the reproductive outcome of patients in each category.

The objective of Dr. Davajan's study was to map out a guideline for counseling patients with similar problems as to their reproductive abilities.

In a 10-year follow-up, none of the patients in the group with polycystic ovary syndrome returned to normalcy and unfortunately some showed a significant increase in body weight, which may have contributed adversely to their medical problems.[1]

In the group with premature ovary failure, replacement therapy with estrogen and progestational compounds is essential. Fertility in this group may be better achieved by ovum donation and embryo transplantation rather than by any heroic attempts at induction of ovulation with high doses of gonadotropin. Spontaneous ovulation, although infrequent, was seen to occur in about 20% of patients studied, and spontaneous pregnancy has occurred while patients received exogenous estrogen therapy.[2]

In the groups with hypothalamic dysfunction and hypothalamic pituitary failure, weight loss and excess physical exercise in eight patients are contributary factors that led to amenorrhea; one patient had anorexia nervosa. Previously published data[3] showed that excess physical activity was responsible in the delay of menarche, irregular cycles, or secondary amenorrhea.

The adverse effect of stress and the beneficial effect of physical rest on the function of the hypothalamic pituitary ovarian axis is well documented. Stressed athletes (runners) were observed to have amenorrhea more frequently than nonstressed ones.[4] Top performer-athletes who participate in sports that require tremendous physical activity and endurance were reported to have a higher incidence of amenorrhea and stress.[5] The role of stress has to be considered when evaluating patients with hypothalamic amenorrhea. An increase in the production of cortisol was seen in patients with hypothalamic amenorrhea when compared with normal controls.[6] In vivo, the administration of corticotropin-releasing hormone resulted in inhibition of LH and FSH secretion.[7] Also, intracerebroventricular administration of corticotropin-releasing hormone was found to reproduce many of the components of the symptom complex of measured depression, hypercortisolism, hypothalamic hypogonadism, anorexia, and anxiety.[8] Physical rest in a group of amenorrheic athletes appeared to lead to resumption of menses without any increase in body weight.[9] Recent studies showed an aberrant pattern of LH pulsation in amenorrheic and cyclic athletes when compared with cyclic sedentary women.[10] In weight loss–related amenorrhea in nonathletes, the pulsatile pattern of LH secretion showed an increased frequency, reduced amplitude, and short duration.[11] Other investigators showed that these patients exhibited abnormal thermoregulation when exposed to low or high external temperatures. Also, partial diabetes insipidus was detected in about one fourth of this group, indicating some form of hypothalamic dysfunction.[12]

In anorexia nervosa the production of corticol was shown to be elevated beyond the normal control group. Recovery from anorexia nervosa was accompanied by about a 30% decrease of the production rate of cortisol.[13] Moreover, adrenocorticotropic hormone–induced adrenal androgen production was found to be impaired at low body weight and that return to normal response occurred after a long-term weight recovery.[14]

The element of depression in anorexia nervosa is another variable to be considered. Measurement of corticotropin-releasing hormone in cerebrospinal fluid in underweight patients and also at intervals after weight restoration, showed elevated levels of corticotropin-releasing hormone that normalized after weight gain. A significant positive correlation was found between cerebrospinal fluid corticotropin-releasing hormone levels and depression scores in weight-corrected patients.[15] Recently it was shown that endogenous opiates play a role in decreasing LH secretion in women with anorexia nervosa. Naloxone infusion led to an increase in pulsatile release of LH in about one half of the patients studied.[16] Induction of ovulation was achieved with naloxone was given daily for 28 days to three women with hypothalamic amenorrhea but without weight loss.[17]

Hypothalamic amenorrhea is a multifactorial disorder. The precise mechanism is not well understood, but a number of factors may contribute to suppression of gonadotropin-releasing hormone pulse generator that will affect the production of gonadotropins. So far, weight loss, excessive exercise, and stress-related amenorrhea, may lead to aberrant production of corticotropin-releasing hormone, cortisol, and β-endorphins, which have been shown to play a significant role in the modulation of the production of gonadotropins and thus may be a causative factor for amenorrhea.

Attempts to correct the underlying predisposing factor in hypothalamic amenorrhea may be the key to a return to normal hypothalamic pituitary ovarian and hypothalamic pituitary adrenal function.

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Dr. Simon R. Henderson, San Francisco, California. Isn't the term *failure* as in hypothalamic-pituitary failure rather a strong term? Because there was such good recovery in that group, shouldn't we perhaps talk about those women as having hypoestrogenic hypothalamic-pituitary dysfunction? How did you treat these patients?

Dr. Kenneth A. Burry, Portland, Oregon. I have chronic battles with my colleagues in medical endocrinology because they like to test everything. Unfortunately for the women in the group with hyperandrogenic chronic anovulation, my tester colleagues have a tendence to want to rule out adult-onset adrenal hyperplasia. How do you approach this problem?

Your experience with suppression of DHEA sulfate in women who take oral contraceptive pills is very similar to our experience with short-term therapy with gonadotropin-releasing hormone analogs. We saw the same reduction in serum levels of this adrenal steroid. What do you think the mechanism might be?

Dr. George Winch, San Francisco, California. This article provides excellent long-term follow-up. I have a patient who had anorexia nervosa diagnosed at age 18 years and had amenorrhea until age 36 years. Having seen her earlier for amenorrhea, I was asked to see her again because of prolonged bleeding. A therapeutic dilatation and curettage revealed endometrial tissue compatible with a well-differentiated endometrial adenocarcinoma. A slide was reviewed by pathologists at the University of California in San Francisco and by Dr. Donald Woodruff of Johns Hopkins University. She was treated with cyclic progestin for 3 months. She has continued to have normal periods for 6 years and repeated curettage procedures about every other year have all revealed normal appearing endometrium. Dr. Davajan, in the small group of patients who started spontaneous cyclic bleeding, did you encounter abnormalities of bleeding? Did you find abnormal endometrium after prolonged periods of unopposed estrogen stimulation?

Dr. Wayne Heine, Tucson, Arizona. This is a very informative article that provides follow-up for 10 years by a single observer. I have two questions (1) In the group with elevated gonadotropin values, did you find an increased incidence of autoimmune problems or other endocrinopathies? (2) Did you test any group for chromosomal abnormalities and if so did you find any?

Dr. Davajan (Closing). Dr. Henderson, I agree that we ought to use terms other than *dysfunction* or *failure* in reference to amenorrhea. I should replace the old terms with *hypoestrogenic* and *euestrogenic amenorrhea.*

All patients in the hypoestrogenic state were treated with estrogen and progestin replacement throughout the entire 10 years unless they wanted to conceive. Six patients regained 22% of their mean body weight and started menstruating when they stopped exercising. The patient with anorexia nervosa underwent psychiatric therapy, regained some of her weight, and achieved a normal cycle.

Dr. Burry, I look for congenital adrenal hyperplasia if the patient's serum DHEA sulfate level is significantly elevated and she has virilization, not just hirsutism and acne. I have been using serum level of 4 μg/ml of DHEA sulfate as my cutoff to look at a 17-hydroxyprogesterone. Although normally I do not diagnose congenital adrenal hyperplasia it is something for which you should be alert. You have to think about the unusual case but the cases in this study were all simple.

Dr. Winch, none of the women who I considered to have made a normal recovery had dysfunctional or abnormal uterine bleeding and biopsy specimens were not obtained. They all had verification of ovulation by serum progesterone and biphasic basal body temperature recordings. I also did not obtain biopsy specimens from patients who had oligomenorrhea, although I recognize that you may get such a patient who shows up with hyperplasia or even early carcinoma. Now that I have the pipelle rather than the Novak curette, I am doing a lot more. I like the pipelle curette because it does not hurt the patient.

Dr. Heine's question about autoimmunity and chromosomes is an excellent one. All seven patients in the hypoestrogenic, hypergonadotropic group were checked for serum antithyroid antibodies and all results were negative. Each of the three patients younger than 25 years had a normal karyotype. At the end of the 10-year study all seven had 24-hour urinary free cortisol looking for early Addison's disease and serum calcium and phosphorus looking for parathyroid involvement. All test results were normal.

Glucose challenge testing in pregnancy

Duncan R. Neilson, Jr., MD, Richard N. Bolton, MD, Robert P. Prins, MD, and Carl Mark III, MD

Portland, Oregon

This study examines results of routine glucose challenge screening in 608 third-trimester patients in a private practice setting. Four patient categories were defined on the basis of the results: normal, abnormal screen with normal glucose tolerance test, borderline glucose tolerance test, and gestational diabetes. When only standard prenatal management practices were used, the fetal outcomes in the four groups were not measurably different. The costs of these procedures resulted in an expenditure of $722.31 per diagnosis of gestational diabetes. This seems excessive in view of the negligible impact of these studies on fetal outcome. Our impression was that routine third-trimester dietary counseling, postprandial glucose monitoring without challenge testing, and evaluation of cases of suspected fetal macrosomia would result in comparable outcome at significantly reduced cost. (AM J OBSTET GYNECOL 1991;164:1673-9.)

Key words: Glucose tolerance, pregnancy, cost-effectiveness, macrosomia

The significance of the diagnosis of gestational diabetes to the pregnant patient and her fetus has been a matter of some concern in our practice. Therefore, in accord with the recommendations of the American Diabetes Association and the American College of Obstetricians and Gynecologists,[1, 2] our office began routine glucose screening of private prenatal patients in 1985.

Before that time we used the established risk guidelines for glucose screening[3]: patients with a history of macrosomia, obesity, intrauterine death, malformations, prior gestational diabetes, glucosuria, or a strong family history of diabetes.

We elected to use the standard 50 gm oral glucose challenge in the nonfasting state with a 1-hour plasma level detected by the glucose oxidase method. We decided to emphasize specificity, at the possible sacrifice of sensitivity, by using a screen level of 150 mg/dl.[2] Those patients with glucose levels ≥150 mg/dl were subjected to a 3-hour oral glucose tolerance test (GTT) after an overnight fast. Actual management of patients was based on postprandial glucose values that were measured in patients with an abnormal GTT. If postprandial glucose values were >125 mg/dl, the patient was given a printed diet sheet outlining an 1800-calorie, 90 gm protein diet. She was retested 1 to 2 weeks later. Only two patients required insulin to keep the postprandial glucose values in the normal range.

We were interested in determining whether the results of standard glucose challenge testing and 3-hour oral GTT correlated with the outcome of the pregnancies, specifically in relation to rates of fetal macrosomia, birth weight, birth trauma, and cesarean section rate. We also were interested in the overall cost of this testing in a private practice setting.

Material and methods

Shortly after delivery we obtained the following data by chart review from 608 sequential patients seen from late 1985 through early 1987: maternal age, gravidity, parity, estimated date of confinement, glucose screen date, delivery date, delivery mode (vaginal or cesarean section), birth weight, glucose screen value, classification of GTT if performed (normal, borderline, abnormal), GTT values, maternal weight at registration, glucose screen time, pregnancy or delivery complications, and Apgar scores. Data were not available in all categories for all patients, but patients were not excluded because of incomplete data, because this reflects the realities of clinical practice. For example, glucose screening values were available for 578 of the 608 patients, and details of delivery were available for 591. For purposes of calculating birth weight distributions and cesarean section rates in relation to the various glucose screening parameters, premature births (<37 weeks) were excluded.

Data were entered into a personal computer spreadsheet program (LOTUS 1-2-3, VIP Technologies), and statistical analysis was carried out with the Lionheart Biometrics (Lionheart Press, Inc., Alburg, Vt.) statistical package. Z, F, and χ^2 distributions were used where appropriate. A value of $p < 0.05$ was considered significant.

Interpretation of GTT results was based on National Diabetes Data Group criteria[4] as follows: fasting, 105 mg/dl; 1 hour, 190 mg/dl; 2 hour, 165 mg/dl; and 3 hours, 145 mg/dl. The test was classified as abnormal if any two values were equal to or in excess of these

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From The Womens Clinic, PC.

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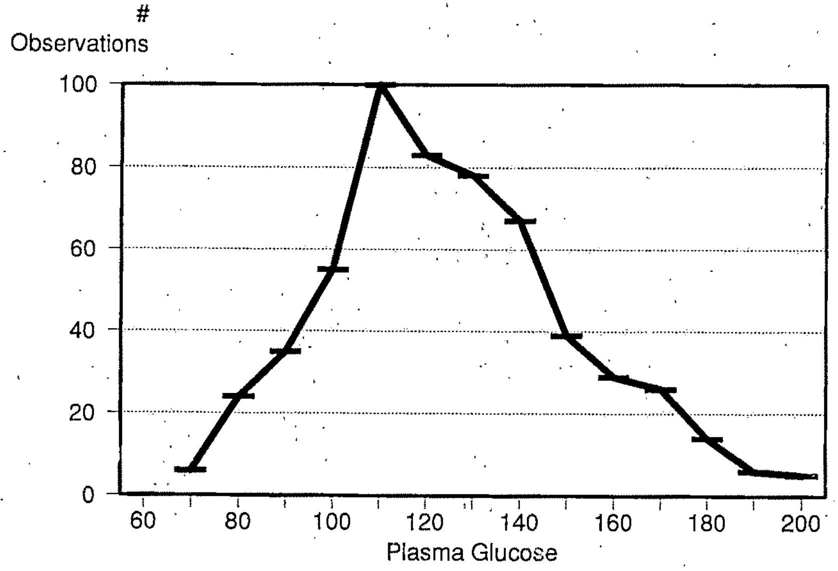

Fig. 1. Distribution of screening glucose values.

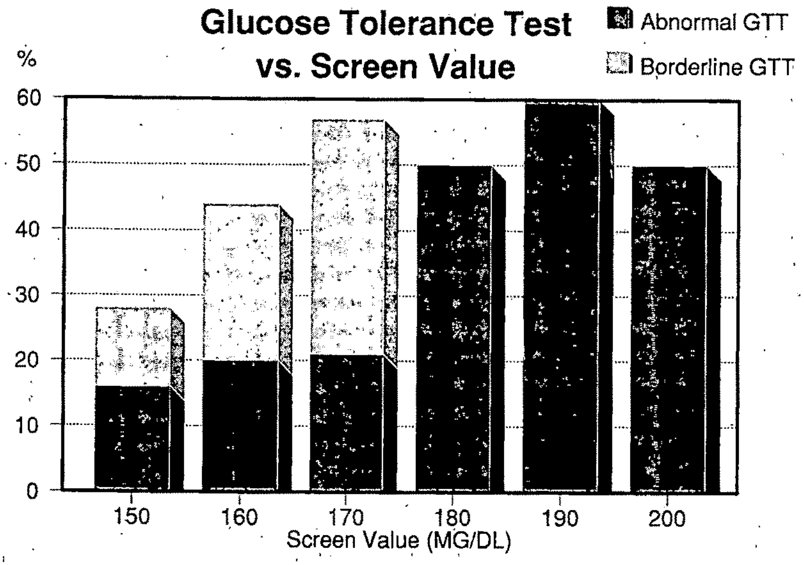

Fig. 2. Percentages of borderline and abnormal GTTs in relation to glucose screen value.

numbers, and the test was considered borderline if any one value was excessive.

Results

Population description. The average maternal age was 27.9, the average gravidity 2.37, and the average parity 0.8. The overall cesarean section rate was 26.7%. After exclusion of breech presentations and repeat cesarean sections, the rate was 18.6%.

Glucose screen results. The distribution of glucose screen values one hour after a 50 gm challenge approximates a normal distribution (Fig. 1) with a mean of 120.7 and standard deviation of 29.0. Thus the value of 150 used as the indication for standard GTT represents 1 SD above the population mean, a value well within the normal population. A value 2 SD above the mean would be 179. Fifteen percent of the population fell above the cutoff value of 150. If 135 had been used as the cutoff, 28% of the population would have required a GTT. If a cutoff of 130 were used, 35% of the population would have required a GTT.

GTT results. Eighty-eight screens were reported as

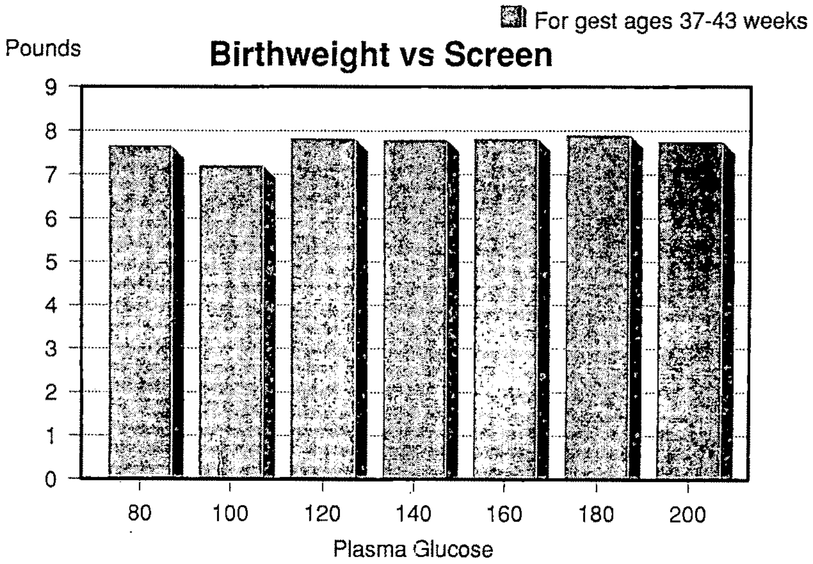

Fig. 3. Birth weight in relation to glucose screen values.

Table I. Birth weight in relation to GTT result

Group	*Birth weight (lb)*		Comparison
	Mean	*SD*	
Whole population	7.69	1.07	Reference
Normal GTT	7.86	1.16	NS
Borderline GTT	7.50	1.15	NS
Abnormal GTT	7.89	0.8	NS

NS, Not significant.

≥150 (15% of the total population). Of these, 83 glucose tolerance tests were performed with the following results: 48 normal, 14 borderline, and 21 abnormal. These results, as well as the frequency of abnormal and borderline GTTs, are shown in Fig. 2.

Birth weights in relation to glucose screening. The birth weights in relation to glucose screen values are shown in Fig. 3. Note that glucose screening values do not predict birth weight in this population. The average birth weight for the entire population is 7.69 pounds, with standard deviation of 1.07 pounds. The birth weights for the subgroups with abnormal glucose screening parameters were not different from this average (Table I).

The distributions of birth weights by GTT category are shown in Figs. 4, 5, and 6. These distributions indicate that the birth weights from the GTT subgroups are not different from those of the population with a normal screen. It is important to note that the gestational ages at delivery were similar for all the subgroups.

Cesarean section rates. Excluding repeat cesarean sections and cesarean sections for breech presentation, the rates of cesarean section delivery for the subgroups are shown in Table II. The cesarean section rates are similar in all groups; i.e., glucose screening parameters did not predict cesarean section delivery rates in this population.

Fetal macrosomia. The numbers of infants weighing ≥4000 gm (8.81 pounds or 8 pounds 14 ounces) are presented in Table III. Glucose tolerance parameters did not predict macrosomia in this population.

Maternal age in relation to glucose screening. Table IV shows maternal age parameters in relation to the glucose screening results. The youngest patient with an abnormal GTT was 22 years of age. Three patients out of the 21 with abnormal GTTs were <28 years old (14%). Only one patient was <24, and 8 patients were <30. Therefore, if age were used as a screening indication, the sensitivities shown in Table V would have been found.

Maternal weight and glucose screening. Table VI

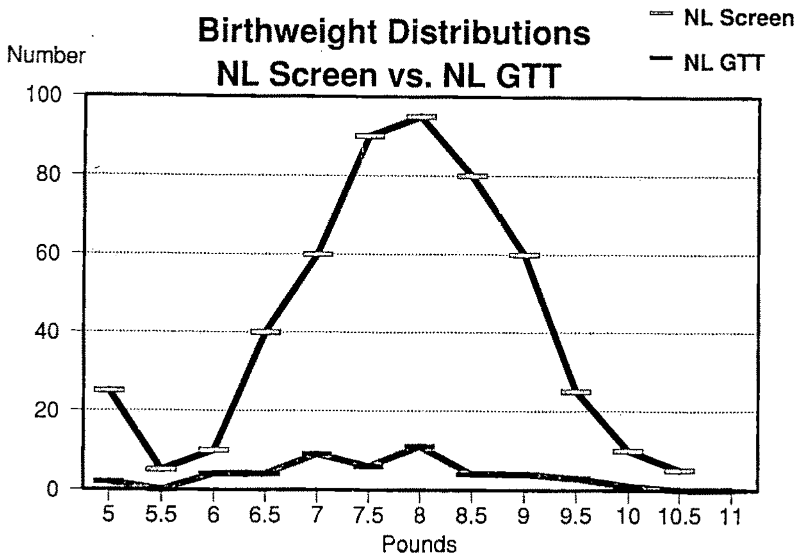

Fig. 4. Birth weight distribution: normal screen versus normal GTT.

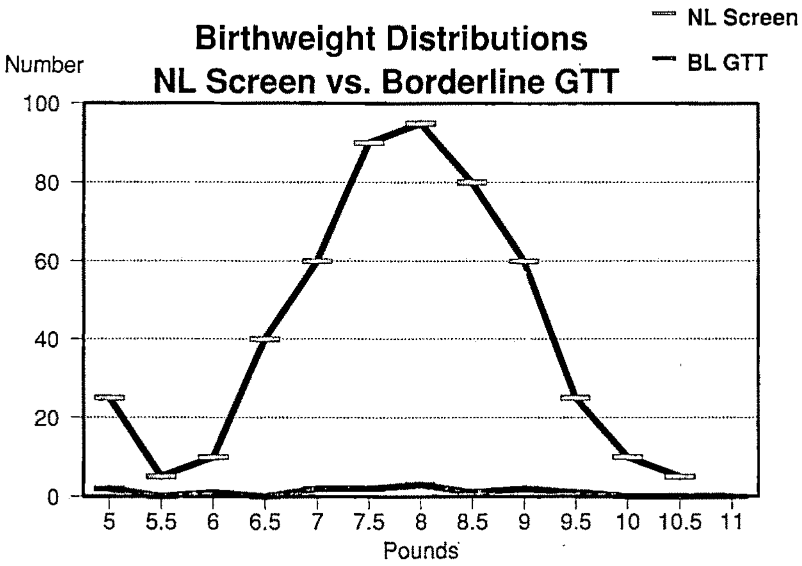

Fig. 5. Birth weight distribution: normal screen versus borderline GTT.

Table II. Cesarean section rate in relation to GTT result

Group	*Cesarean section rate*
Whole population	18.6%
Normal GTT	21%
Borderline GTT	15%
Abnormal GTT	20%

shows the maternal weight values in relation to glucose screening parameters. Maternal weights did not predict abnormal screening results or results of GTTs. If anything, the patients with abnormal glucose tolerance gained less weight in the third trimester than did the others. This may have reflected dietary counseling, which was systematically done only in the third trimester.

Comment

It would not be expected that the minimal management used in this study would completely eliminate pathologic conditions associated with abnormal glucose metabolism in pregnancy, and therefore the results of this study suggest that standard glucose challenge testing practices do not predict these conditions (fetal macrosomia, birth trauma, cesarean section delivery) with enough sensitivity to be cost-effective in a routine private practice setting. Instead, dietary advice given

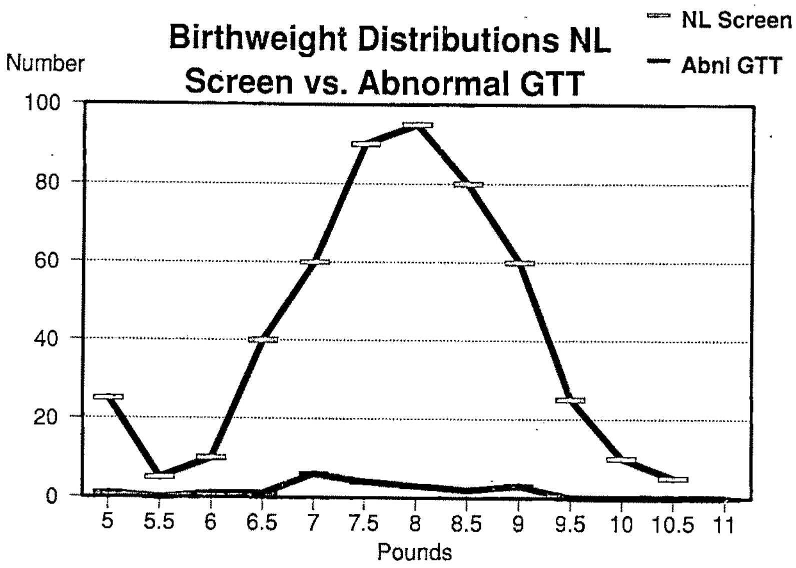

Fig. 6. Birth weight distribution: normal screen versus abnormal GTT.

Table III. Fetal macrosomia (birth weight >4000 gm) in relation to GTT result

	Macrosomic infants			
Group	*Total No. of patients*	*No.*	*%*	*Expected No.*
Screen <150 mg/dl	501	70	14	74.1
Normal GTT	48	8	17	7.1
Borderline GTT	14	1	7	2.1
Abnormal GTT	21	4	19	3.1

Based on rate for whole population.

to patients with elevated postprandial blood glucose levels in the third trimester might seem to eliminate the need for challenge testing. This concept is supported by the work of Lind.[5] Birth trauma did not occur in this study, and there were no significant neonatal complications.

Weingold et al.[2] state that routine glucose testing is cost-effective but do not substantiate the concept with cost per test or cost per case or cost per case of gestational diabetes diagnosed. Swinker[6] reports a cost per patient screened and a cost per case diagnosed as $10.00 and $173.00, respectively, using 130 mg/dl as the cutoff value for 3-hour glucose tolerance testing. Marquette et al.[7] report a cost of glucose screening to be $2.45 per patient, and GTTs to be $11.00 in a 1985 study. Lavin[8] reports a cost of $4.75 per patient screened and a cost of $328.96 per case of gestational diabetes detected. Our cost was $17.75 per screen and $59.15 per GTT, giving our patients a total cost of $10,259.50 for 578 screens, $4904.00 for 83 GTTs, and a cost per diagnosis of gestational diabetes of $722.31. This cost would be significantly higher if we had chosen a lower cutoff value for the 1-hour screen.

Table IV. Maternal age in relation to glucose screen result

Group	*Average age (yr)*
Screen <150 mg/dl	27.5
Normal GTT	30.8
Borderline GTT	32.9
Abnormal GTT	30.5

Table V. Screening sensitivity in relation to maternal age

Age (yr)	*Sensitivity (%)*
≥24	95
≥28	86
≥30	62

One might cut costs by limiting the screening to older patients, but costs would still be high and accuracy questionable. If, as Marquette[7] suggests, only patients ≥24 years of age were tested, we would have a sensitivity of

Table VI. Average maternal weight versus glucose screening result

Group	*Initial weight (lb)*	*Screen weight (lb)*	*Delivery weight (lb)*	*Total gain (lb)*	*Third-trimester gain (lb)*
Normal screen	140.7	164.7	171.8	31.1	7.1
Normal GTT	142.9	162.5	173.2	30.3	10.8
Borderline GTT	143.1	162.2	169.4	26.2	7.2
Abnormal GTT	142.2	159.4	164.5	22	5.0

95%. He reported a sensitivity of 83% with this age criterion. If only patients >30 years were tested, we would have a sensitivity of 62%.

Elevated maternal weight and excess weight gain were not predictors of abnormal glucose tolerance, increased birthweight, or cesarean section rates.

Jovanovic and Peterson[9] report that 27.7% of their patients with a glucose screen >150 mg/dl at 27 to 31 weeks' gestation were delivered of infants weighing ≥4000 gm. Our number was 15.2% for all patients with a glucose screen >150 mg/dl, which was comparable to the rate of 14% in patients with a screen of <150 mg/dl.

In conclusion, the results of this study cast doubt on the current approach to gestational diabetes. We have been led to believe that challenge testing will help in the management of pregnancies and that therapies based on these tests are beneficial. This study does not support this concept. Fetal weight, morbidity, difficult delivery, and maternal morbidity were not different in the various groups defined by glucose challenge testing. Costs of challenge testing are substantial and probably do not contribute to improvement of fetal or maternal outcome.

We feel that postprandial glucose measurement at the time of other routine blood testing in pregnancy would cost less and give us the physiologic values we actually use to manage patients. Routine third-trimester dietary counseling and aggressive induction of labor for postdate pregnancies or suspected fetal macrosomia might help eliminate the pathologic conditions associated with gestational diabetes.

We do not feel, as did Hunter and Milner,[10] that "gestational diabetes is a diagnosis looking for a disease" but rather that gestational diabetes is a disease looking for a diagnosis.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. JOHN ENBOM, Corvallis, Oregon. This study group involved data on 608 obstetric patients in a 2-year period given a 50 gm, 1-hour, postprandial glucose challenge and, further, a 100 gm GTT in those with values found to exceed 150 mg/dl on the screen. Eighty-three (15%) had an abnormal screen and were compliant in follow-up; 48 (7.9%) studies were normal, 21 (3.5%) were abnormal, and 14 (2.3%) were borderline. Gestational length appeared to relate to macrosomia, and advancing maternal age with an increasing incidence of operative delivery. No birth trauma, fetal morbidity, or neonatal hypoglycemia was noted.

The resulting study group, 21 patients with the diagnosis of gestational diabetes out of 608 screened patients, points out the need for a larger patient population or collaborative studies to evaluate the multiple factors related to morbidity and mortality of gestational diabetes. I do find it interesting that maternal weight gain was reduced in the third trimester after diagnosis and the initiation of a diabetic diet; this points out the importance of motivation when the patient is confronted with a diagnosis that presents potential risk to her fetus.

The authors chose a screening level of 150 mg/dl as their abnormal cutoff, to increase specificity in the screened group. This level may have validity, but unless a lower screening value such as 135 to 140 mg/dl is used to substantiate the specificity and sensitivity of a group, I don't feel one can rely on the assumption that this screening level will not miss a substantial number of patients with gestational diabetes. The fact that the screening level was 1 SD above the mean does not answer the question of the total incidence of gestational diabetes in a screened population.

In 1964, when O'Sullivan and Mahan[1] first published their data on pregnancy-induced diabetes, there was definite need for diagnostic criteria for risk management in prenatal care. The contribution of a standardized method of diagnosis and management for gestational diabetes has been invaluable. The continuing importance of this diagnostic area in risk management has been emphasized again in an updated *ACOG Technical Bulletin* from the American College of Obstetricians and Gynecologists,[2] recommendations from the Centers for Disease Control in 1986[3] and the Second International Workshop Conference of Gestational Diabetes Mellitus in 1985.[4] In all guidelines the emphasis is on diagnostic criteria and, after this diagnosis, a management scheme.

Cost in diagnosis and management are important areas that I feel were brought out in this paper and need to be further addressed. The cost of diagnosis in this article was $722.37 per case of gestational diabetes and would have been higher if a lower screening value had been chosen. This is essentially twice the figure stated in Everett's article on screening for gestational diabetes.[5] It would have been beneficial to see the cost figures for screening levels of 135 and 140 mg/dl as well. Everett suggested further research should be focused on decreasing the cost of screening for gestational diabetes. Wouldn't it be better to screen all patients in a more cost-effective and clinically significant manner?

If patients who maintain a normal fasting and 2-hour postprandial blood glucose have a low perinatal mortality rate[6] and postprandial glucose studies after normal dietary intake may be more physiologic, it follows that evaluation of the parameters of treatment is a more effective and cost-efficient method, as suggested by Schneider and Cevert.[7] They have suggested the term pregnancy-induced glucose intolerance and use of a postprandial level of 120 mg/dl. Other authors have suggested similar screening of postprandial blood glucose levels for diagnosis and follow-up of patients on diet management.

The question of the later predictive value of the current gold standard in diagnosing diabetes mellitus, the 100 gm GTT, has also been brought into question by Naylor,[8] who suggested only a 36% positive predictive value for this diagnosis after delivery in a highly selective group with glucose intolerance during and after pregnancy and the need to consider higher values in the diagnosis of gestational diabetes that may correlate with future diabetes mellitus in a more predictive manner.

With increasing costs and reduced relative health dollars, we must increase our efficiency in the cost of diagnosis and apply more stringent criteria for the clinical diagnosis of gestational diabetes.

I would like to ask Dr. Bolton the following: (1) Do you still feel that a screening value of 150 mg/dl is the most effective cutoff value, regardless of the cost of screening? (2) Were all newborns screened for hypoglycemia or only those that appeared symptomatic? (3) How does your group currently screen for gestational diabetes in your office population? Would you suggest different guidelines?

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Dr. Bolton (Closing). I am very pleased to have not been bombarded with technical questions from the perinatologists.

Dr. Enbom, our thesis is that the cutoff value used for screening, at least in the ranges we tested, does not seem to make much difference. Although some obstetricians in Portland are now using 130 or 135 mg/dl, it would cost more to do GTTs on women whose screening tests are at that level. As a result of this study, we will no longer screen women younger than age 24. We will, however, monitor postprandial blood glucose values on all mothers throughout pregnancy. In this study the blood glucose values were checked only for babies who were symptomatic, and we tracked these through the pediatricians. The babies got along quite well.

The problem in monitoring gestational diabetes is we are on the outside looking in. We indirectly study the fetus by checking maternal glucose, hemoglobin A_{1c}, and fructosamine, while what we really need to know is the level of fetal insulin. Of course, we do not have a very good handle on that because insulin, being rather uncooperative, does not cross the placenta. A recent study[1] from Graz, Austria, reports a large number of amniotic fluid insulin determinations. It clearly shows that when fetal insulin is normal there is no problem. The group of gestational diabetic patients in whom the fetus has elevated insulin concentrations is the problem. We need to find a practical, noninvasive method to discriminate between the two groups, but for the moment, we do not have it.

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Alternative liability insurance: A physician-owned captive insurance company

George F. Lee, MD
San Francisco, California

The physician-owned captive insurance company is a lesser known but dynamic alternative to commercial insurance. The Physicians Reimbursement Fund, Ltd., was founded in 1975 in response to the malpractice crisis of that year. The company insures about 100 physicians in high-risk specialties. Approximately one half are obstetrician-gynecologists. Innovative management has enabled this company to operate successfully at a fraction of the premium charged by typical insurance companies. Fourteen years of experience have demonstrated the ability of this company to successfully serve the needs of the community. (AM J OBSTET GYNECOL 1991;164:1680-5.)

Key words: Liability insurance, captive insurance company, physician-owned insurance company

In 1975 both the cost and availability of medical liability insurance reached crisis proportions in California. Commercial insurance carriers withdrew from the marketplace, leaving many communities in Northern California with no alternative for malpractice insurance.

One solution to this problem was the formation of "doctor-owned" insurance companies. These companies were able to restore availability of malpractice insurance to the physician community. Cost, however, remained a problem for some specialties. This has been particularly true for the field of obstetrics.

Currently, commercial carriers only offer claims-made policies. Claims-made policies, in contrast with occurrence policies, may require the purchase of a tail when a physician decides on a career change. As a result of these financial considerations, some obstetricians have chosen to go "bare." This option is not in the best interest of the patient or the physician. On the positive side, California's 1975 Medical Injury Compensation Reform Act has had a profound effect on stabilizing the previously escalating costs of liability insurance. California's legislative reform package has become a model for the rest of the United States.

Alternative solution

A less understood alternative to both the commercial market and the doctor-owned companies is the "captive insurance company." By definition, a captive insurance company is a wholly owned or controlled subsidiary company formed primarily to insure the risks of its noninsured parents or affiliates. An "offshore captive" is incorporated in a foreign jurisdiction and operates out of that foreign location while primarily insuring the domestic risks of its parent. The parent may be an individual, a company, or an organization of individuals with a common insurance need.

The Physicians Reimbursement Fund, Ltd., is an offshore captive insurance company incorporated in the Cayman Islands of the British West Indies in 1975. This company serves the interests of its founding physicians, who are approximately 100 high-risk specialists who practice in the City of San Francisco. About 50% of the physicians insured by this company are obstetrician-gynecologists. As a result, the management and experience of this company reflect the experience of this specialty.

The impetus to form the company arose early in 1975 when it appeared that there would be no viable insurance carriers in the San Francisco Bay area. The main objectives were and remain: (1) ensure availability of malpractice insurance; (2) contain costs; (3) embody the principles of California's 1975 Medical Injury Compensation Reform Act; and (4) offer an occurrence-based policy. A guiding principle of this insurance company is the mandatory requirement for all participating physicians to use binding arbitration.

After a careful analysis, Grand Cayman of the British West Indies was chosen as the site of incorporation. The offshore site offered a number of advantages including minimal capital requirements, minimal regulatory requirements, a stable economic and political history, readily accessible air travel, and professionals schooled in the formation of captive insurance companies.

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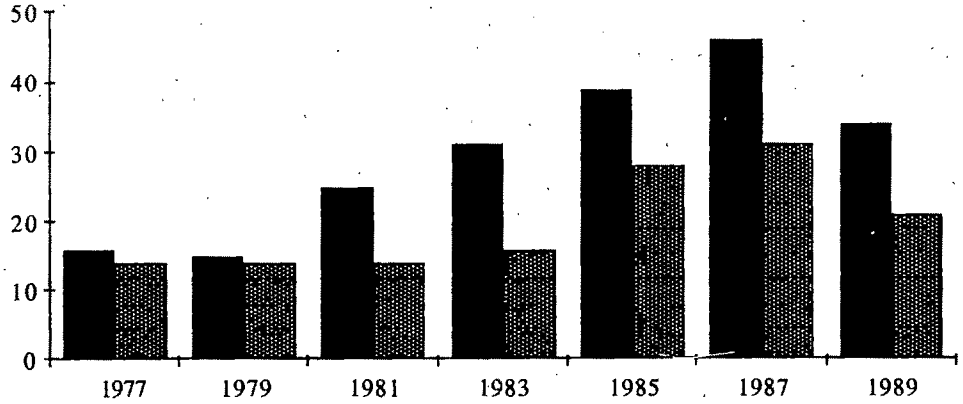

Fig. 1. Physicians Reimbursement Fund, Ltd., claims experience. *Solid box,* Incidents reported; *stippled box,* claims and active cases.

Organizational format

The relationship between the onshore and offshore components of an operating captive insurance company is moderately complex. Whereas the Physicians Reimbursement Fund, Ltd., is incorporated and performs all its insurance duties at the offshore site, management of claims and educational support to the physicians are performed in San Francisco through the auspices of the Patient Protective Association. The Patient Protective Association is a California nonprofit corporation. It acts as agent for those insured. To comply with California regulations it is essential to separate the various functions and compartmentalize them according to state requirements. Appropriate legal, accounting, and actuarial services are readily available to management in day-to-day operations. In this capacity management uses the same guidelines as any domestic insurance carrier with respect to underwriting and investment policies. A board of directors comprises four physicians, three of whom are members of this Society, and provides all the management services for the company. On the basis of the requirements of the 1986 Tax Reform Act, the company elected to be treated as a U.S.-based insurance company for tax purposes.

Premiums

The Patient Protective Association, acting as agent for the physicians, collects all premiums and transmits them to the insurance company in Grand Cayman. Each physician pays an initial premium of $2000, and during the first 12 years paid an annual premium of $6000 ($500 per month). The limits of insurance during those years was $250,000 per occurrence and $600,000 per year. All physicians regardless of specialty were treated equally. In 1987 the limits of insurance were increased to $500,000 and the premium increased to $12,000 per anum ($1000 per month). At this time, all premiums paid to this company remain on deposit as reserves for future claims. To date, all operating expenses, settlements, and awards have been paid out of the earnings from the company's reserves. The Physicians Reimbursement Fund, Ltd., is managed by four physician members of the Board of Directors. Legal counsel in Grand Cayman provides office space and secretarial support staff. All funds are deposited in Bank of America, Grand Cayman. Accounting services are provided by Coopers and Lybrand, Grand Cayman. Actuarial services are provided by The Wyatt Company, San Francisco. The total cost of managing the insurance company is approximately $100,000 per year.

Claims management

The Patient Protective Association provides educational support services and undertakes to manage claims on behalf of the insured physician.

The office of the Patient Protective Association is located in San Francisco. Staffing for the Patient Protective Association requires one part-time bookkeeper and a full-time chief executive officer. Total operating costs for managing the Patient Protective Association are approximately $90,000 per annum. These funds are derived from dues paid by the physician members of the Patient Protective Association. Each member is assessed $100 per month.

Claims experience

Claims experience for the Physicians Reimbursement Fund, Ltd., has been remarkably predictable over the past 14 years. From 1976 to 1987 there was a gradual increase in the number of incidents reported. This reflects an increased awareness of the physician members and their willingness to assist the company in performing its services. Physicians are encouraged to report any incident that might eventually turn into a legal action. Actual claims have likewise shown a gradual increase

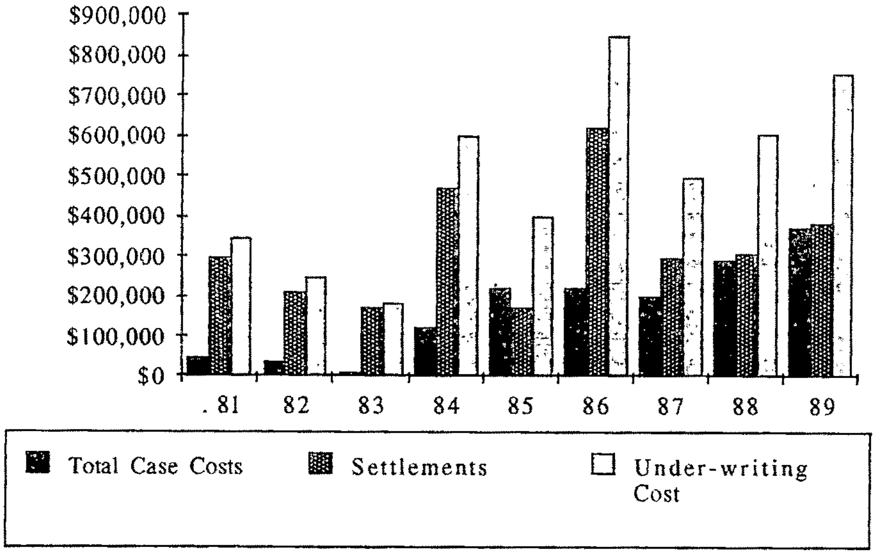

Fig. 2. Physicians Reimbursement Fund, Ltd., major claims losses.

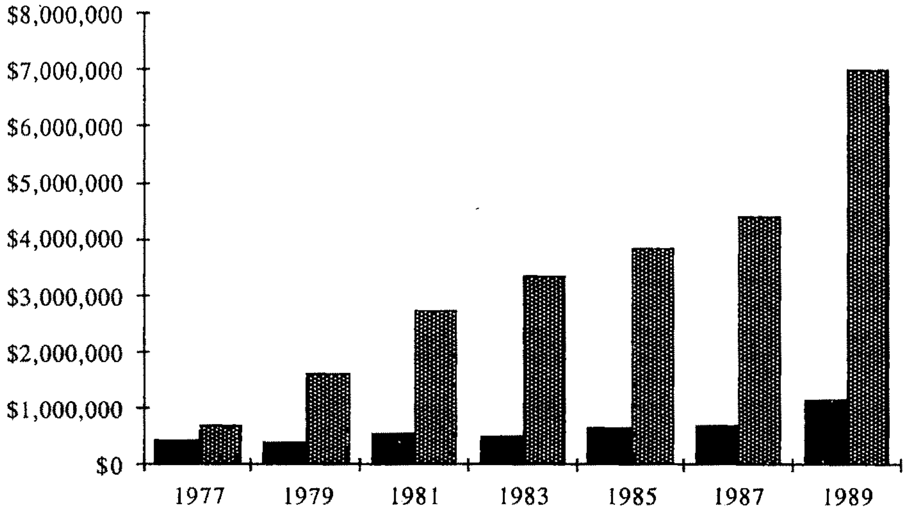

Fig. 3. Physicians Reimbursement Fund, Ltd., premiums paid and reserves. *Solid box,* Premiums paid; *stippled box,* reserves.

through 1987 and a subsequent softening during the past 3 years. These trends are comparable to the national experience (Fig. 1). Total case costs, settlements, and underwriting costs have slowly increased over the life of the company. This reflects increases in filed claims and legal costs (Fig. 2). The incidence of claims filed against physicians in this company is about equal to that of the national average. Despite this fact, the company has been able to service the needs of the physicians and the patients at a substantially reduced premium. It is our estimate that over the life of this company, the average obstetrician has saved approximately $260,000 in premium costs. The ultimate beneficiary is the consumer, our patient.

Total reserves for the company are approximately $9 million. This represents about $100,000 per insured physician, and compares very favorably with the commercial carriers in California.

Management principles

Management believes that the success of this company is a result of careful adherence to the provisions of the Medical Injury Compensation Reform Act, including the vigorous use of arbitration. In addition, there are three unusual features of this company's operations.

1. Peer visibility. All those insured work in the same community. Each insured is expected to donate time to the organization for claims review or committee work. This tends to promote a high level of consciousness concerning suit prevention.

2. Code green. Physicians are encouraged to intervene actively in suit avoidance and problem resolution. At a physician's request, the company will reimburse a patient for economic loss as a result of complications or maloccurrence (i.e., excess hospital costs, home care, lost wages, repair of dental crown). No release is required from the patient.

3. In-house case management. Most of the company's claims are handled by a claims committee composed of physicians. Very few cases are handled by legal defense firms, and when they are the progress of the case is monitored very closely, including the need for depositions and expert reviews.

By virtue of an aggressive approach to settling cases, only four cases have gone to arbitration and two cases to trial during the 14-year history of the company. All but one of these cases were won by the defense.

Comment

The Physicians Reimbursement Fund, Ltd., is an example of a small but effectively run offshore captive insurance company. In a volatile market it guarantees availability and has shown an ability to control costs. Whereas it is not commonly understood, there are many physician groups, hospitals, and clinics across the United States that successfully use this model to provide liability insurance. At a time when many obstetricians face escalating insurance costs, a captive insurance company is a viable alternative.

Special consideration is given to Ms. Linda Osborne for her creative assistance.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. Keith P. Russell, Los Angeles, California. I have two questions: (1) How many physician-owned captive companies are there in the United States? I know of a number owned by organizations, but I am inquiring about pure physician-owned companies. (2) What is your application process for physicians? Is there a selection or can anyone join?

Dr. Russell K. Laros, San Francisco, California. Are payments made to patients by your company exempt from the new national legislation that requires reporting to a national data bank? Are you concerned that with the requirement for reporting any sum given to patients, physicians will be less willing to make payments and settlements? Do you anticipate growing reluctance on the part of your physicians to participate in code green?

Dr. Theodore W. Loring, Eureka, California. I congratulate Dr. Lee and his associates for designing and forming this alternative liability insurance company for high-risk physicians, especially obstetrician-gynecologists. Dr. Lee told us the reasons for selecting the Grand Caymans—minimal regulatory and capital requirements, a stable economy and political history, and professionals schooled in the formation of captive insurance companies. These professionals have been well compensated for their efforts in the offshore captives.

The period 1975 to 1980, coinciding with our California malpractice crisis, was an era of experimentation in providing insurance. The exodus of commercial companies, not the physician-owned companies, caused introspection by physicians from which came many different forms of insurance. Before that time, claims-made insurance did not exist. The offshore captive insurance plans became numerous. In contrast with Dr. Lee, I believe that captives are not widely distributed throughout the United States, and that there have been many failures among them. Many people formed captives, nonphysicians and unfortunately some physician entrepreneurs. After a time many of these companies folded, but the investors still made a lot of money. This is because in the malpractice insurance business few claims of significance surface before 3 to 5 years after a policy is issued. Were this Society, for example, to form a captive company and begin to collect premiums today, it would be 3 or 5 years before we would have to pay any significant amount of money in claims. During the interval, our income could be invested and from investment income we could pay small claims. This is just what the physician's captive in San Francisco has done.

Physicians and their patients both need protection. Therefore when an insurance company is licensed by most states it must meet certain requirements. In California we have had quite a few companies fail since 1975, but there is a state Insurance Guarantee Fund to which every licensed insurance company must contribute. Every company licensed to sell insurance in the state—medical malpractice, automobile, homeowners, or whatever—is assessed to be sure that people who need protection will get it should a company fail. The fund relieves the physician of liability if an insurance company fails. Offshore captives do not have to meet these requirements.

This company provides coverage on an occurrence basis rather than a claims-made basis, and this gives the physicians who belong to it some long-term protection. In 1975, stock companies left California in droves and there are now just two that function in California. Stock companies sell their stock to the public and must make a profit to be successful. If by chance they have a bad year, either in underwriting or in investments, premiums for the next year are increased. Profits, when they do occur, go to stockholders, not to the insured physicians. The stockholders of a mutual company, on the other hand, are the insured. Profits, if any, are distributed to the insured as dividends. In California, insurance exchanges have a mix of features of stock and mutual companies. In addition, there are cooperatives that are not insurance companies and therefore are not controlled by the state insurance laws.

Dr. Lee's company, which insures 90 to 100 physicians in high-risk specialties, would not be appropriate for the general medical population. Despite insuring those at high risk, the company succeeds because it is run by a very dedicated group of people. Physician involvement in management, particularly on the board, and in underwriting and claims management are important factors. They review the insured physicians as peers. In looking at a claim, the doctor can easily envision himself or herself in the defendant's position.

I am surprised that you have been able to maintain a $250,000 claim limit until 1987 and $500,000 since then. You must have had charmed lives during a period of megaawards in cases involving infants with neurologic deficit. Fortunately, in the past 3 years, doctor-owned companies in California have had a great claims experience. In 1989 and again in 1990, premiums were reduced, even for obstetrics. The company with which I am involved expects a significant further decrease in 1991.

Dr. Lee, I have several questions about your presentation: (1) The number of members has remained about the same or declined a bit since 1985. Is this because of underwriting rejections? (2) Are the claims shown in Fig. 3 still open or have they been closed? For example, for 1983 the chart shows approximately five claims and 10 active cases. Is this a total of 15 cases? (3) In Fig. 4, does the total underwriting cost include the total case costs and settlement costs, or just the underwriting costs? (4) You mention a 14-year history of success with arbitration. Why did you have two cases that went to trial? My understanding is that when the patient signs a binding arbitration agreement, she must accept the decision of the panel.

Dr. Carl Goetsch, Berkeley, California. As president of the California Medical Association in 1975, I am a bruised and battered survivor of the professional liability crisis. In 1950 I began to do case evaluation and defense for a program underwritten by American Mutual Insurance that covered all the Northern California counties. Some years the company lost a little money, but on average they came out very well. Two or three years before the crisis of 1975, the premiums rose sharply. Basically a workmen's compensation company, they pulled out of the professional liability business when the insurance commissioner of Massachusetts said they must put more in surplus. Therefore I think it is important to consider a program's long-term history before you evaluate its efficacy. Your company is successful, at least it is still viable. Nonetheless, we are not yet in a position to evaluate this method of coverage as a particularly good or bad way of approaching the problem. For one thing, your group is so small that the law of averages has not had full play. Dr. Lee, is the contract for this company assessable?

Dr. Lee (Closing). One tenet basic to the formation and operation of this company was to assure the availability of insurance. When we first developed the idea, the commercial carriers had withdrawn and the doctor-owned companies had not started. Over the years, pride of ownership on the part of most of the physicians in the group has maintained the company.

Dr. Russell, I do not know how many captive companies are owned by physicians in the United States. We are closely allied to six companies in California and I am aware of a number of other companies through our attorneys in the Grand Caymans. My reading in the *Wall Street Journal* about the Harvard Community Hospital program, one of the largest programs formed in the Grand Cayman Islands, was what prompted my investigation of offshore captives. After we looked at the Bahamas and other places in the Caribbean and Europe, we selected the Grand Caymans because of the good reputation it had from people who had actually worked with the people there.

Dr. Laros, you hit on my most worrisome management problem for the next 10 years. The federal government will soon require the reporting of any money paid on behalf of the patient. Fortunately, we think there will be two exceptions. First, you can write off your bill, even a big one. Second, we can reimburse for out-of-pocket spending. There is also a gray zone that will allow individual companies to interpret what is reportable. Attorneys from many companies including ours are working hard to establish criteria. What we have done with the code green concept is to make the patient satisfied and not worried about who is right or wrong. It works and is a valuable part of what we do. Its loss would put us, and all of medicine, in jeopardy. I think we will retain some of it because our message will be heard at the national level.

Dr. Loring, it is true that a captive insurance company can be set up for inappropriate purposes, retirement plans or tax shelters. It is also true that if a captive insurance company fails, people are left in the cold, as with any business. We do not participate in the California insurance fund. Because this is a doctor-owned company, each of the owners has full knowledge of its statistics. The insight that each person has and the ability of each to participate, contributes to management's decisions with respect to ensuring that there are adequate reserves. This serves as a major force for the company to do what is in the best interest of the physician owners. If we were to close the company tomorrow and take no more premiums over the next 8 years (i.e., the statute of limitation), we could probably live off of the earnings from the company and still return to all the physicians every dollar they ever put into it. In this company there is probably a higher percentage of reserves per doctor than in most commercial companies. Because we do not have a large company to back us up if we go bankrupt, management is intense and it is imperative that we maintain a high level of reserves.

The most important mark of our company is that physicians participate in the decision making and contribute to how the company operates. Because they are aware of the day-to-day operation of the company and know what is happening in the marketplace, it is not likely that the company is going to leave people high and dry.

The intensity with which the group responsible for physician selection looked at each other's risk level was extremely high when we started in 1975. Since then we have had no interest in growing in numbers. It is only to serve the interests of the physician group that the company exists. When a member retires, a replacement is drawn from the medical community, perhaps someone joining an existing practice. Most are relatively fresh from residency and have no track record that can be used to assess them. Our experience over the past 15 years has not been remarkably different from the national experience in terms of suits and claims filed.

You mentioned that it may take 5 years for claims to surface. In 15 years we have had only one claim surface more than 12 months after the incident and that was at about 14 months. We have been able to predict our future losses accurately. Because there is a statute of limitations in it, the arbitration contract probably has a role in making these claims surface early. However, I reemphasize that we do stand alone. It is not a perfect system and there is a bit of risk in it.

You noted our limits. The structured award part of current California medical liability law is extremely important to us. A structured award of $200,000 put on the table today results in $3.2 million over the life span of an injured child. In California, the total multimillion dollar award is not the cost to the insurance company as it is in New Jersey, for instance, where the award is paid up front. The fact that in California the award is paid on a monthly basis over the life span allows us to have a lower limit and still service a higher payout.

Dr. Goetsch, each physician is assessable for $6000. When we started the company we needed a guarantee from each person to pay 1 year's premium, $6000 in case we had miscalculated our costs over the next 5 years. We have not needed to collect it over the 15 years. Being assessable for $6000 is still written into the contract and there has been no problem with acceptance by new members. If the company should go bankrupt, we would need that amount to satisfy outstanding obligations. Thus although we would hate to pay it, the money would be there to pay on our behalf for the company's obligations.

LETTERS TO THE EDITORS

Residency training and abortion

To the Editors: The article on obstetric ethics and abortion (Chervenak FA, McCullough LB. AM J OBSTET GYNECOL 1990;163:1425-9) is an interesting presentation on ethical issues relating to abortion. In closing the authors discuss the implications for obstetric residency training programs. Whereas I agree that no resident should be forced to perform abortions, training in the technique of abortion should be a standard component of obstetrics and gynecology residency training programs. Currently, relatively few programs require abortion training; most simply make such training available on an optional basis for those who request it. Given that abortions in the United States are performed primarily by obstetrician-gynecologists, it is imperative that all be trained in this procedure. We do not give residents the option as to whether they will be trained in the technique of vaginal or abdominal hysterectomy or of any other procedure commonly performed by obstetrician-gynecologist specialists.

Clearly, there must be an option to allow those who truly are opposed to abortion for religious or other reasons not to have to perform the procedure. However, for all others it should be required and should be listed as another required procedure when programs are reviewed by the Residency Review Committee. It is imperative that obstetrician-gynecologists be trained, particularly in second-trimester procedures, because they are the only physicians who will be doing these more complex procedures.

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Dilatation despite cervical cerclage: A diagnostic pitfall

To the Editors: The risks of uterine contractions in the presence of cervical cerclage are laceration of the cervix and rupture of the uterine corpus. We observed a case of a paracervical uterine rupture with extension to the vaginal fornix, presenting as if it were an effaced and dilated cervix.

A 24-year-old gravida 4, para 0, abortus 3 woman had a Shirodkar procedure in week 19 of pregnancy because of cervical incompetence in association with twin pregnancy. At 27 weeks' gestation, the patient was admitted to the hospital with painless vaginal bleeding. Ultrasonography excluded placenta previa. Biamniotic twins were seen: the lower child in breech presentation and the other in transverse lie. Speculum examination and vaginal palpation did not demonstrate the presence of the suture, and 3 cm cervical dilatation was felt. Some hours after admission, premature contractions started and an intravenous ritrodrin treatment was instituted.

In spite of tocolysis progression of labor was noted. The membranes ruptured spontaneously and dilatation proceeded to 7 cm. Cesarean section was performed after induction of general anesthesia. The first infant, a girl in breech presentation, weighed 910 gm and had Apgar scores of 7 and 8 at 1 and 5 minutes, respectively, and an umbilical artery pH of 7.31. The second infant, also a girl, weighed 900 gm, had Apgar scores of 6 and 7, an umbilical artery pH of 7.33, and was extracted in breech presentation after version from transverse lie. Both infants were immediately transferred to the neonatal intensive care unit.

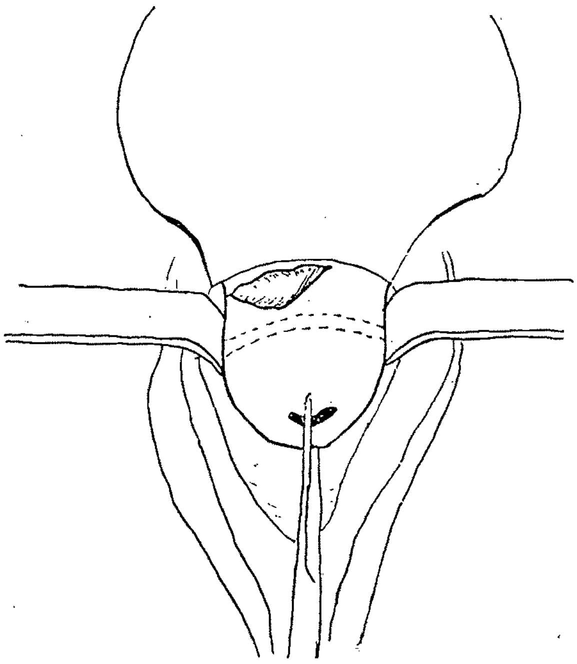

Fig. 1. See text for explanation.

At surgery it became evident that the cervix was still closed with an intact ligature, but there was a 6 cm paracervical uterine rupture extending from the attenuated lower posterior segment to the left lateral vaginal fornix (Fig. 1). Some minor oozing from the edges of the lesion was noted. At vaginal examination this rupture must have been considered an almost completely effaced and dilated cervix. The site of the uterine rupture was abdominally sutured, thereafter the forniceal lesion was closed transvaginally and the ligature was removed. The patient recovered well; the infants' neonatal development was favorable.

To our knowledge this is the first reported case of paracervical uterine rupture resulting in a false way to the vagina in the presence of a closed cervix with the ligature in place. The only more or less comparable case was reported by Pelosi et al.[1] They describe a successful vaginal delivery through a preexisting cervicovaginal fistula. There was no preexisting fistula in our patient. The reported incidence of cervical laceration after cerclage has been performed varies from 1% to

13%.[2] Interpretation of the vaginal examination created some specific difficulties: careful inspection did not show the ligature or its knot and it was not found at palpation. The cervix was compressed against the upper vaginal wall and the rupture presented as if it were a centrally positioned, dilated cervix. The patient showed none of the classic clinical signs and symptoms of uterine rupture.

When a patient known to have a cervical cerclage presents in preterm labor and effective tocolysis cannot be achieved, the ligature must immediately be removed. If it is impossible to visualize the ligature in the presence of dilatation, a paracervical rupture must be considered before deciding the suture has broken.

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Cytomegalovirus infection

To the Editors: I agree with Drs. Grose and Weiner (Grose C, Weiner CP. Prenatal diagnosis of congenital cytomegalovirus infection: two decades later. AM J OBSTET GYNECOL 1990;163:447-550) that congenital cytomegalovirus infection is a major health problem in need of more attention in the perinatal literature. They conclude that amniotic fluid culture for cytomegalovirus is a useful adjunct when congenital cytomegalovirus infection is suspected. The question is what should be used to raise one's suspicion?

Maternal cytomegalovirus infection, as in two of the authors' cases, may be an acceptable indication for amniocentesis. A second indication is a fetus with ultrasonographic evidence of central nervous system abnormalities—especially because such patients would usually be offered amniocentesis for chromosome analysis anyway. Thus there would be no further potential for procedure morbidity by the addition of a cytomegalovirus culture to the fluid analysis.

Unfortunately, as the authors point out, cytomegalovirus infection in adults, pregnant or not, is usually not significantly symptomatic and therefore is uncommonly diagnosed. Similarly, most fetuses with intrauterine cytomegalovirus infection probably have no abnormalities that are detectable by ultrasonography, although this needs further study. As with toxoplasmosis, the vast majority of suspected congenital infection would be identified only by routine maternal serologic screening.

There are significant differences, however, between cytomegalovirus and toxoplasmosis with regard to those fetuses infected in the first half of pregnancy. Whereas the majority of those infected with toxoplasmosis will have significant postnatal sequelae, only 10% of neonates with positive cytomegalovirus cultures have adverse sequelae. More important, toxoplasmosis therapy has proved efficacy, whereas cytomegalovirus antiviral therapies do not.

Despite the high prevalence of cytomegalovirus in the community, routine maternal serologic screening (the only proved way to identify the vast majority of cytomegalovirus infection) is not generally recommended. This should change with the development of drugs effective against herpes viruses.

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Intravenous immunoglobulin treatment of recurrent pregnancy loss and antiphospholipid antibodies

To the Editors: We read with great interest the review by Reece et al. (Reece EA, Gabrielli S, Cullen MT, et al. Recurrent adverse pregnancy outcome and antiphospholipid antibodies. AM J OBSTET GYNECOL 1990;163:162-9). We would like to comment on another possible mode of treatment in these patients by intravenous immunoglobulins.

Immunoglobulins play a central role in immune regulation. Intravenous preparations of immunoglobulin have become available for clinical use in a wide range of diseases including treatment of patients with recurrent abortions of the immunologic type.

Several reports have been published concerning the treatment of recurrent pregnancy loss as a result of antiphospholipid antibodies with intravenous high-dose immunoglobulins[1-4] and are summarized in Table I.[5] Determination of treatment intervals and doses used in these studies were empiric because phospholipid antibody tests did not appear to be useful to monitor therapy. This is probably why the patients were also treated with low-dose heparin and aspirin.

The effectiveness of this new mode of therapy may be related to several immunologic mechanisms such as blockade of antibody binding to receptors on macrophages or antiidiotypic interactions. Preliminary data concerning the efficacy of intravenous immunoglobulin in the treatment of this condition are encouraging but further studies are required to determine the optimal mode of therapy.

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Table I. Intravenous immunoglobulin treatment for recurrent abortions because of antiphospholipid antibodies[5]

Reference	No. of patients	No. of previous abortions	Therapeutic regimen				
			Steroids	*Aspirin*	*Heparin*	*Ig*	*Mode of Ig administration*
Carreras et al.[1]	1	9	—	—	—	400 mg/kg	Three courses at 17, 22 and 27 weeks' gestation
Scott et al.[2]	1	9	60 mg/dl	80 mg	—	400 mg/kg	Two courses at 8 and 14 weeks' gestation
Parke et al.[3]	1	5	—	80 mg	5000 U twice a day	600 mg/kg	Monthly infusion from 6 months before conception until delivery
Wapner et al.[4]	1	4	—	80 mg	Unreported dose	1000 mg/kg	Monthly doses from 9 to 34 weeks' gestation
	1	2 + [2]*	—	80 mg	Unreported dose	1000 mg/kg	Monthly doses from 10 to 33 weeks' gestation

Ig, Immunoglobulins.
*Two electively terminated pregnancies.

tibodies and Rh immunization. AM J OBSTET GYNECOL 1989;159:1055-6.
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Response declined

Follow-up of elevated maternal serum α-fetoprotein levels: Ultrasonography only or amniocentesis?

To the Editors: Because of current discussions that amniocentesis may not be necessary for patients with an elevated level of maternal serum α-fetoprotein (AFP) and a normal level II sonogram,[1, 2] we reviewed the experience in our laboratory in 1989. We receive maternal serum AFP and amniotic fluid specimens for prenatal diagnosis from a broad referral base in the greater metropolitan area of Atlanta.

Fourteen cases of spina bifida–meningomyelocele and encephalocele (collectively referred to as neural tube defects) were detected prenatally during 1989. Anencephaly was excluded from this review because the diagnosis by ultrasonography alone is not in question. Two patients (cases 1 and 2) each had elevated maternal serum AFP levels (6.0 and 3.0 multiples of the mean) followed by a normal level II sonogram at 17 levels and 18 weeks' gestation, respectively. Amniotic fluid AFP was 4.2 and 2.2 multiples of the mean, and each had a positive acetylcholinesterase value. On the basis of these abnormal results, a second high-resolution ultrasonographic evaluation was performed on case 1, and a second and third were performed on case 2 before spina bifida was detected in both fetuses. A third patient (case 3) had a normal sonogram performed before amniocentesis at 16 weeks' gestation because the patient had insulin-dependent diabetes. Amniotic fluid AFP level was 2.1 multiples of the mean and the acetylcholinesterase value was positive. On the basis of this abnormal result, the level II sonogram was obtained and spina bifida was detected. Case 4 had a maternal serum AFP level interpreted as not elevated (2.1 multiples of the mean) at 16 weeks' gestation, normal "routine" sonogram, and no amniocentesis; spina bifida was detected by ultrasonography near term.

Therefore sensitivity for initial detection of fetal neural tube defects by ultrasonography without amniocentesis at this referral laboratory was 10 of 14 or 71% during 1989. In our experience the incentive to "look harder" appears to be based in part on the abnormal results from the amniocentesis. Current medical practice emphasizes dependency on amniocentesis. In our series, seven patients (cases 5 through 11) with pregnancy ages ranging between 15 and 34 weeks, had isolated neural tube defects detected by ultrasonography before amniocentesis. All these had elevated amniotic fluid AFP levels or positive acetylcholinesterase values, confirming what was already known, and all chromosome study results were normal. Another patient (case 12) with an elevated maternal serum AFP level was found by ultrasonography to have hand deformities as well as encephalocele; fetal chromosome diagnosis was requested. Chromosomes were normal, amniotic fluid AFP level was 5.7 multiples of the mean, and the acetylcholinesterase value was positive. Only the remaining two patients (cases 13 and 14) had elevated maternal serum AFP levels at 18 and 20 weeks' gestation, respectively, and a neural tube defect was detected in each fetus by ultrasonography alone with no amniocentesis, the scenario recommended by Nadel et al.[2]

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HCG or β-hCG or hCG β-subunit or hCG + hCG β-subunit?

To the Editors: I wonder whether authors, reviewers, and editors of prestigious obstetric and gynecologic journals are concerned about what has been measured when determinations of choriogonadotropin or its subunits are published.

According to the title and text of one of the latest articles in this respect (Schiff E, Ben-Baruch G, Moran O, et al. Prediction of residual trophoblastic tissue in first-trimester abortions and low levels of human chorionic gonadotropin β-subunit. AM J OBSTET GYNECOL 1990;162:797-801), the determination of human chorionic gonadotropin (hCG) β-subunit was intended. However, the method to which the authors referred is characterized as follows: "The capture antibody . . . binds both intact hCG and its beta-subunit. . . . we used two ^{125}I labeled antibodies. . . . One of these . . . binds intact hCG, the other . . . is specific for the beta-subunit of hCG"[1] and "We calibrated the standards against IRP 75/537,"[1] which contains complete hCG essentially without free β-subunit.[2] Thus by the method Schiff et al. quoted, complete hCG + hCG β-subunit is measured and the result is expressed in terms of IU (IRP 75/537), although "this . . . approach can be adapted to measuring intact hCG and its beta subunit either separately or in combination."[1] Schiff et al., on the other hand, state "The measurement units are according to the second international standard of the International Health Organization." However, this standard, IS 61/6, "is unsuitable for use in all immunoassay systems because of its heterogeneity"[3] or at least is subject for discussion.[4]

In any case, neither IRP 75/537 nor IS 61/6 but IRP 75/551 (or hCG β of the CR series) is appropriate as standard for measurement of hCG β-subunit.[2] In addition, Schiff et al. do not mention whether or in which way they modified the method[1] for measuring not hCG + hCG β-subunit but the β-subunit separately.

Finally, they do not discuss whether or why in the intended prediction of residual trophoblastic tissue, measurement of hCG β-subunit should be advantageous as compared with measurement of hCG or with measurement of hCG + hCG β-subunit. This advantage seems to be rather improbable because the molar ratio of free hCG β-subunit to intact hCG was found to be about 1/100 in the first half of pregnancy.[5, 6]

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Reply

To the Editors: We appreciate the comments by Dr. Kuss regarding our article. His concern with respect to the method used is understandable. The method reported by Rattle et al.,[1] to which we referred, can indeed be adapted to the measurement of intact human chorionic gonadotropin and its β-subunit, either separately or in combination. We have again verified with our laboratory that the assay used for all our blood samples specifically tested the β-subunits of human chorionic gonadotropin by the same method (i.e., iodine 125 immunoradiometric assay) involving specific anti-β-subunit monoclonal antibodies.

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Simplified urethrocystovaginal suspension for stress urinary incontinence

To the Editors: I am very interested in the article by Varner (Varner RE. Retropubic long-needle suspension procedures for stress urinary incontinence. AM J OBSTET GYNECOL 1990;163:551-7). I described practically the same procedure (Cervicocystovaginopexie simplifiée dans le traitement de l'incontinence urinaire [Simplified cervicocystovaginopexy for stress urinary incontinence] Montpellier Chir 1972;18:191-3). The only difference is the vaginal incision. I used a midline incision and not a transverse incision (Fig. 1, *A* and *B*).

In 1978, Peyreyra reported the blunt retropubic dissection already published in my article.

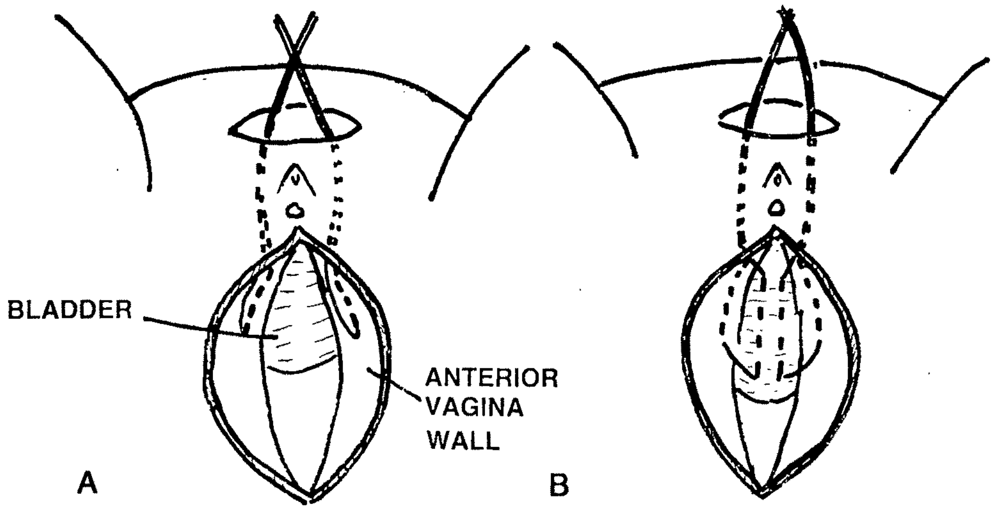

Fig. 1. A, Ureterovaginal suspension. **B,** Ureterocystovaginal suspension.

In 1982, Muzsnai published the same technique with a little modification: He used three sutures placed through the vaginal wall with one bite instead of one suture with several bites as in my procedure.

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Reply

To the Editors: Dr. N. Nghiem Toan has noted two further articles in the French literature that describe procedures that use variations of Dr. A.J. Pereyra's original procedure. These were overlooked as references in my article. I apologize for this. I was able to review Dr. Nghiem-Toan's article with a French translator.

Dr. Nghiem-Toan described a variation of the modified Pereyra procedure that he performed on eight patients with "very nice results for 18 months." He used a single-needle passage from the rectus fascia downward to each side of the bladder neck as was described by Pereyra. Unlike the current Pereyra procedure, he apparently did not penetrate or dissect the space of Retzius from below, but "dissected up to the level of the anterior pubic rami." There was no mention of cystoscopic observation of the anterior bladder to rule out suture perforation, which may be a somewhat common occurrence when the needle is passed without digital guidance. Finally, a vertical vaginal incision was used as was mentioned in the above correspondence.

The procedure described in the August 1990 issue of this JOURNAL is unique only in that it uses parts of several previously described procedures to optimize results without increasing operative time and potential morbidity. The suture-loaded Stamey needle is passed from the vaginal to abdominal fascia through the space of Retzius twice on each side of the bladder neck after incorporation of "paravaginal fascia" in three bites. It is believed that the separate passage of the suture ends, some 1½ cm apart, may further serve to support the bladder neck by thus incorporating pubourethral connective tissue in the support. Cystoscopic observation of the baldder neck to rule out suture perforation and to note proper bladder neck elevation is performed. The transverse vaginal incision is considered to be optimal in that there is no traction on the vaginal suture line. Suture line traction may occur when sutures elevate the vaginal wall lateral to a vertical incision at the level of the bladder neck.

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Should routine liver biopsy be done for the definite diagnosis of acute fatty liver of pregnancy?

To the Editors: Dr. Barton et al. presented a case of recurrent acute fatty liver of pregnancy (Barton JR, Sibai BM, Mabie WC, Shanklin DR. Recurrent acute fatty liver of pregnancy. AM J OBSTET GYNECOL 1990;163:534-8) in which they recommended routine liver biopsy for electron microscopy as a means to establish the definite diagnosis of acute fatty liver of pregnancy.

We do not agree with their opinion because of the following reason. Acute fatty liver of pregnancy, which can only be diagnosed histologically with the use of liver specimen in definition, and the hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome, which can be diagnosed by noninvasive blood test, share many clinical signs and laboratory abnormalities, especially elevated liver enzyme values and coagulation problems. On the basis of our previous article[1] we believe that one cannot differentiate histologically between acute fatty liver of pregnancy and the HELLP syndrome, and we believe that both diseases are the same disorder. HELLP syndrome has been recognized as a disease that requires immediate delivery to improve clinical outcome.[2-4] Even if the definite diagnosis of acute fatty liver of pregnancy is made for a patient who already shows several signs of HELLP syndrome in addition to no reasonable explanation for the disease excluding HELLP syndrome, treatment may not be altered and one has little to offer a patient in exchange for liver biopsy findings. Further, because our article[1] raises the question of whether acute fatty liver of pregnancy is an independent clinical entity, we do not think that electron microscopy allows us to establish the definite diagnosis of acute fatty liver of pregnancy. Also, we emphasize that liver biopsy is not necessary, at least in clinical practice.

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Reply

To the Editors: We appreciate the interest of Drs. Minakami, Takahashi, and Tamada in our article. We do not suggest routine liver biopsy as a means of establishing the definitive diagnosis of acute fatty liver of pregnancy in all patients for whom this diagnosis is entertained. Rather, if a liver biopsy specimen is obtained for evaluation in these patients, a sample should be properly prepared for electron microscopic analysis as well as routine microscopic examination.

We agree that there is overlap, both clinically and pathologically, between acute fatty liver of pregnancy and hemolysis, elevated liver enzymes, and low platelet count (HELLP) syndrome. A concurrent diagnosis of HELLP syndrome in patients with biopsy-proved acute fatty liver of pregnancy has been reported.[1] But we believe that these syndromes can be distinguished histopathologically.[2] On light microscopy, the vacuolization and necrosis is most prominent in the central zone in acute fatty liver of pregnancy, whereas in HELLP syndrome the necrosis is periportal. On electron microscopy, although fat droplets may be present in hepatocytes from patients with HELLP syndrome, fat droplets are much more numerous in acute fatty liver of pregnancy.

Therefore we continue to believe that liver biopsy is a useful tool to confirm a diagnosis of acute fatty liver of pregnancy. Because of clinical and laboratory variations, this diagnostic procedure is infrequently necessary in the differential diagnosis of HELLP syndrome versus acute fatty liver of pregnancy.[3] Further, the treatment of both conditions remains delivery of the fetus. However, a liver biopsy is useful in distinguishing acute fatty liver of pregnancy from other forms of fulminant hepatic failure that do not require delivery, such as fulminant herpes simplex hepatitis.[4]

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Wright, Francis McGill, Jr., 2115 Far Hills Ave., Dayton, OH 45419
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New certifications: Subspecialty of Reproductive Endocrinology

Date of examination: December 1990

Place: Chicago, Illinois

Number certified this examination: 39

Cumulative total of certificates awarded for this subspecialty: 407

Bayer, Steven Robert, 3 Seal Harbor, #431, Winthrop, MA 02152
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Cruz-Burgos, Rosa Ileana, 282 J.T. Pinero Ave., El Amal, Suite 205, Rio Piedras, PR 00927
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New certifications: Subspecialty of Maternal-Fetal Medicine

Date of examination: December 1990

Place: Chicago, Illinois

Number certified this examination: 59*

Cumulative total of certificates awarded for this subspecialty: 618

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Brustman, Lois Eileen, 3 Westwood Ct., Harrison, NY 10528
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Chan, Linda, Jefferson Med. Ctr., 1025 Walnut St., Room 310, Philadelphia, PA 19107
Cherouny, Peter Herbert, 24 Laurel Dr., Hershey, PA 17033
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Darby, Marilyn Jean, 46 Treaty Dr., Wayne, PA 19087
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Moretti, Michael Louis, Maternal-Fetal Medicine, 853 Jefferson, Suite E102, Memphis, TN 38103

The names of eight newly classified diplomates are not included in this listing at their request.

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Owen, John, Dept. Ob/Gyn, Univ. Station, Univ. Alabama, Birmingham, AL 35294
Pastorek, Joseph G., II, Louisiana State Univ., 1542 Tulane Ave., Ob/Gyn, New Orleans, LA 70112
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Penso, Christine Arety, 3 Andrew Circle, Natick, MA 01760
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Rajabi, Mohammad Refai, 5100 O'Bryan, Montreal, Quebec, Canada H4V 2A7
Reece, E. Albert, Yale Univ. School Med., 333 Cedar St., Ob/Gyn, New Haven, CT 06510
Rodis, John F., 182 South Road, Farmington, CT 06032
Ronkin, Sheila L., 300 Cherry Bend, Merion Station, PA 19066
Saller, Devereux N., Jr., 63 Pocono Dr., Warwick, RI 02888
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Sloan, Chris T., 932 Forest, Birmingham, MI 48009
Towers, Craig V., 16412 Wishingwell Lane, Huntington Beach, CA 92647
Ward, Sheila Marie, 201 W. Chapel Ridge Road, Pittsburgh, PA 15238
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New certifications: Subspecialty of Gynecologic Oncology

Date of examination: December 1990

Place: Chicago, Illinois

Number certified this examination: 26*

Cumulative total of certificates awarded for this subspecialty: 400

**The name of one newly classified diplomate is not included in this listing at his/her request.*

Ashraf, M., 200 Wedgewood Dr., #103, Morgantown, WV 26505
Baker, Vicki Vaughan, (526) MSB 4415, Gyn. Oncol., Univ. Cincinnati, Ob/Gyn, Cincinnati, OH 45267
Bryson, S.C. Peter, Dept. Ob/Gyn, Kingston Gen. Hosp., Kingston, Ontario, Canada K7L 2V7
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Rowley, Kenneth Coit, 5001 David Ct., Cincinnati, OH 45215
Schink, Julian C., Prentice Women's Hosp., 333 E. Superior, #420, Chicago, IL 60611
Smith, Lloyd Herbert, 1309 Aspen Pl., Davis, CA 95616

Books received

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ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

The Harvard Medical School, Department of Continuing Education, and The Massachusetts General Hospital, Department of Gynecology, are presenting a postgraduate course in "Vaginal Surgery," directed by David H. Nichols, MD, FACS, FACOG, on October 11, 12, and 13, 1991, at the Ritz-Carlton, Boston, Massachusetts. For information contact: Harvard Medical School, Department of Continuing Education. Tel.: (617) 432-1525.

The Center for Study of Multiple Birth will sponsor the first Twins Days Research Grant Competition and award a $1000 prize to a researcher who proposes the most significant research study. The competition is limited to citizens of the United States and its territories and is open only to qualified researchers who study twins. A requirement for the prize winner is that he or she agree to publish the research in a scientific journal. Interested applicants must submit a typed 250-word abstract of the proposed research to the judging committee, at The Twins Days Festival Committee, Inc., P.O. Box 29, Twinsburg, OH 44087, not later than July 1, 1991. The award will be announced by The Festival Committee and the Center for Study of Multiple Birth on Aug. 3, 1991, at the Twins Days Festival, Twinsburg, Ohio.

Obstetric/Pelvic Imaging, Hands-On Instruction in Diagnostic Ultrasound. Conducted August 12-16, 1991, in Dallas, Texas. For further information call or write Keith Mauney & Associates, Inc., 600 East Las Colinas Blvd., Suite 250, Irving, TX 75039. Tel.: (214) 402-8250.

Third Congress of the International Society of Gynecological Endocrinology, Madonna di Campiglio (Italian Alps), February 9-16, 1992. The presentations will relate to the topic, "The Hormones in Gynecological Endocrinology: From Synthesis to Clinical Implications." For further information contact: Prof. Andrea R. Genazzani, President of I.S.G.E., Department of Obstetrics and Gynecology, University of Modena, Via del Pozzo 71, 41100 Modena, Italy. Tel.: 39 59 360255-360224. Fax: 39 59 371401.

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Illinois

Carle Clinic Association is seeking a Board Certified or Board Eligible **Perinatologist.** Carle Clinic Association is a 210 physician, multi-specialty group practice in central Illinois providing services to an area of 1.3 million residents in 42 counties.

Carle Foundation Hospital and Covenant Medical Center have cooperative Level II Neonatal Centers and are presently pursuing Level III designation. Each organization has two Neonatologists.

Compensation is by means of salary with bonus eligibility after the first year. Extensive fringe benefits include malpractice coverage, outstanding retirement package, health, life and disability insurance and excellent vacation and meeting time.

If interested in this opportunity contact Robert C. Parker, Jr., M.D., Medical Director, Carle Clinic Association, 602 West University, Urbana, Illinois, 61801, (217) 337-3417.

DOOR COUNTY, WISCONSIN

BC/BE Obstetrician/Gynecologist to join established BC OB/GYN in hospital - based practice. Complete practice facilities available. New LDRP Birthing Center in modern, progressive 89 - bed community hospital. Competitive guaranteed salary and incentive package. Malpractice insurance. Attractive benefits.

Door County offers exceptional four season recreation along Lake Michigan shores, proximity to Milwaukee and Chicago, top rated schools and quality community life for family.

Send CV to: **Richard C. Murray, M.D.**
330 South 16th Place
Sturgeon Bay, WI 54235

Gynecologic Oncologist
Faculty Position

The James H. Quillen College of Medicine of East Tennessee State University (ETSU) is seeking a board certified/eligible Gynecologic Oncologist. This position offers an excellent opportunity combining research, teaching and private practice with a faculty position at the Assistant/Associate Professor level. Salaries are very competitive and fringe benefits are excellent. The Medical School is located in the Tri-Cities area of Northeast Tennessee in a beautiful section of the Appalachians and within seeing distance of the Blue Ridge Mountains. The area has excellent hunting, fishing, and camping with multiple ski resorts at your doorstep. The Tri-Cities combine good schools and rural living in a safe, beautiful environment. Yet, the medical referral base consists of 1.3 million people within a 50 mile radius of the Medical School. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman, Dept. OB/GYN
James H. Quillen College of Medicine
East Tennessee State University
P.O. Box 19570A
Johnson City, TN 37614-0002

ETSU is an AA/EOE

Geisinger Medical Cente

This 577 bed Medical Center located in Danville, Pennsylvania is the largest rural tertiary referral center in the country. The 275 physician hospital based group practice represents 63 specialties, 16 accredited residency programs and offers basic and clinical research facilities.

OB/GYN

The division of 9 OB/GYN physicians offers career opportunities for an additional BC/BE specialist in Maternal-Fetal Medicine and a Generalist OB/GYN with strong obstetrical commitment.

- **1200 deliveries**
- **23 bed level III nursery**
- **Accredited residency program**
- **University affiliation**
- **Attractive call schedule**

Geisinger offers competitive compensation plus a generous benefit package including four weeks vacation, four weeks CME with funding, malpractice, health, life and disability insurance, retirement plan and relocation expenses. Send CV to: **Robert L. Walker, M.D., Geisinger Medical Center, Danville, PA 17822-1329.** Equal Opportunity Employer M/F/H/V.

Geisinger

NORTHERN CALIFORNIA
BAY AREA
PERINATOLOGIST

Outstanding practice opportunity for BE/BC perinatologist to join solo private practice. The Good Samaritan Hospital is a private, level III center with over 4000 deliveries annually. NICU is staffed by five neonatologists. Excellent institutional support with full range of prenatal diagnostic services. Experience desired in CVS, prenatal diagnostic ultrasound, PUBS and complicated pregnancies. The Antepartum Testing unit is state of the art. San Jose is located in the South Bay 40 miles south of San Francisco. Exceptional lifestyle and financial package. If interested call (408) 559-2258 or send Curriculum Vitae to:

Kevin J. Gomez M.D.
2425 Samaritan Drive
San Jose Ca. 95124

OBSTETRICIAN/
GYNECOLOGISTS

Group Health Association, one of Washington, D.C.'s most respected names in the health maintenance field, has immediate and future openings for board certified or eligible Obstetrician/Gynecologists.

Consisting of nine multi-specialty medical centers and 200 physicians, Group Health's highly skilled support staff serves over 150,000 members. This staff attends to the details of clinical administration, allowing you the freedom to devote your energy to practicing medicine. Further, GHA's challenging work environment offers a diverse patient population and affiliation with the area's finest hospitals.

A generous benefits package including vacation, sick leave and insurance coverage (malpractice, life, retirement, disability and dental) awaits you in return for your expertise. In addition, you can look forward to company-paid hospital and licensing fees, professional society dues, continuing education as well as predictable office hours.

If you would like more information, please send your CV. to the address below or call Mary Hines, Physician Recruiter, on 1-800-336-1442 or in DC Metro area, (202) 364-7442.

Turner Bledsoe, M.D., Medical Director

Group Health Association
4301 Connecticut Avenue, N.W.
Washington, D.C. 20008
An Equal Opportunity Employer

OB/GYN POSITIONS

Minneapolis, Minnesota

The Department of Obstetrics and Gynecology at Hennepin Faculty Associates (HFA) is seeking additional qualified academic physicians in **Maternal-Fetal Medicine, Reproductive Endocrinology, and Generalists** with special interests in other disciplines of our specialty.

Candidates must be board certified or active candidates for certification. Salary is competitive and a comprehensive fringe benefit package is provided.

Positions will have clinical and teaching responsibilities at Hennepin County Medical Center and academic rank will be established at the **University of Minnesota**.

Please contact:

Stephen H. Cruikshank, M.D., Chairman
Department of Obstetrics and Gynecology
Hennepin County Medical Center
701 Park Avenue South
Minneapolis, Minnesota 55415

Telephone: (612) 347-2750

HENNEPIN
FACULTY
ASSOCIATES

Hennepin Faculty Associates is an Equal Opportunity/Affirmative Action Employer

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OB/GYN
TO BUILD PRACTICE

Progressive, 188-bed medical center, located in a beautiful Southeastern Kansas university town, has great need for another OB/GYN. Because of over 500 deliveries in the service area, the two OB/GYN's and Family Practice physicians cannot meet the demand for both Obstetrics and Gynecology. The 28-physician medical staff will welcome you, and the hospital will do everything possible to help establish your practice. Build this fine practice while enjoying a quality lifestyle in a college town. Outstanding financial package. Send CV or call:

Mark Hanna
Jackson and
Coker, Inc.
115 Perimeter
Center Place
Suite 380 10720
Atlanta, GA 30346
Tel. 1-800-544-1987

JACKSON
AND COKER

Generalist Obstetrician/ Gynecologist

Faculty Position

The James H. Quillen College of Medicine of East Tennessee State University (ETSU) is seeking a board certified/eligible Generalist Obstetrician/ Gynecologist. This position offers an excellent opportunity combining research, teaching and private practice with a faculty position at the Assistant/Associate Professor level. Salaries are very competitive and fringe benefits are excellent. The Medical School is located in the Tri-Cities area of Northeast Tennessee in a beautiful section of the Appalachians and within seeing distance of the Blue Ridge Mountains. The area has excellent hunting, fishing, and camping with multiple ski resorts at your doorstep. The Tri-Cities combine good schools and rural living in a safe, beautiful environment. Yet, the medical referral base consists of 1.3 million people within a 50 mile radius of the Medical School. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman, Dept. OB/GYN
James H. Quillen College of Medicine
East Tennessee State University
P.O. Box 19570A
Johnson City, TN 37614-0002

ETSU is an AA/EOE

Southern
California

Join The Partnership of Choice

Southern California Permanente Medical Group (SCPMG), the nation's largest multi-specialty medical partnership, has been providing quality healthcare for nearly a half century. Our continued growth has created new practice opportunities for:

OBSTETRICIANS & GYNECOLOGISTS

You'll have a choice of professional environments ranging from a 250-bed community hospital to a major teaching facility in a number of prime Southern California locations. Our active practices require proficiency in modern ultrasound and surgical techniques.

We offer a **highly competitive salary, paid malpractice, predictable hours and much more.**

Send your curriculum vitae to **Irwin P. Goldstein, M.D., Associate Medical Director, SCPMG, Dept. 006, Walnut Center, Pasadena, CA 91188-8013.**

Or call: **1-800-541-7946**

KAISER PERMANENTE
Southern California Permanente
Medical Group

Partners Practicing Good Medicine

Chairman, OB/GYN

Long Island Jewish Medical Center (LIJ), is searching for a Chairman of its Department of Obstetrics and Gynecology. The Medical Center is an 825-bed tertiary care institution with over 40 fully approved residency training programs and fellowships and a total house staff complement of over 550 residents and fellows. LIJ has major responsibilities for medical student teaching in all required clinical clerkships. Fully accredited Obstetrics and Gynecology residency training program; large full-time and voluntary faculty.

The Chairman of Obstetrics and Gynecology is responsible for the professional staffing and services at the Medical Center and at our municipal hospital affiliate, the Queens Hospital Center, a 540-bed general hospital. Candidates must be Board Certified with proven experience as a clinician, educator and investigator and should qualify for academic appointment at the level of Professor at the Albert Einstein College of Medicine.

Please submit curriculum vitae in confidence to David R. Dantzker, MD, Chairman of the Search Committee for Obstetrics and Gynecology, Chairman, Department of Medicine, Long Island Jewish Medical Center, New Hyde Park, NY 11042. *An Equal Opportunity Employer.*

LIJ Long Island Jewish
Medical Center

The Long Island Campus for the
Albert Einstein College of Medicine

Obstetrician/ Gynecologists

Marshfield Clinic, a 350 physician multispecialty group practice is seeking BE/BC Obstsetrician/Gynecologists to join expanding regional referral centers in Chippewa Falls and Woodruff/Minocqua, Wisconsin. These are beautiful four-season recreational locations combining professional excellence with a family-oriented life style. Both have newly constructed Clinics with comparably modern hospitals. These practices provide an opportunity to enjoy the autonomy of a private practice with the financial and professional resources of a major multispecialty group. **Chippewa Falls** is located 1 1/2 hours from downtown Minneapolis/St. Paul and borders Eau Claire, Wisconsin, a city of nearly 80,000 which includes a major 4 year campus of the University of Wisconsin. Known as the "Lakeland Area," **Woodruff/Minocqua** is truly one of the most attractive vacation areas in the upper Midwest. An emphasis on quality practice and life style is combined with a very competitive salary and one of the finest fringe benefit packages in the country. For additional information, please send C.V. and references to: **David L. Draves, Director Regional Development, Marshfield Clinic**, 1000 North Oak Avenue, Marshfield, WI 54449, or call 1-800-826-2345, extension 5376.

MARSHFIELD CLINIC

OB/GYN

A 180-bed County Hospital located in the San Francisco Bay Area and serving a Medi-Cal and HMO population is seeking a BE/BC OB/GYN. Duties will include active GYN surgery service and outpatient GYN and Prenatal Clinics; supervision of U.C. Davis-affiliated residents in a 150 delivery/month OB service. Night call will be shared with four others and can be taken from home. Competitive salary and benefits. Send C.V. to: **D. Kent Hobert, M.D., Department OB/GYN, Merrithew Memorial Hospital, 2500 Alhambra Avenue, Martinez, CA 94553.**

Equal Opportunity Employer

Merrithew
Memorial
Hospital
AND CLINICS

OB/GYN
WISCONSIN

Multi-specialty group in suburban Milwaukee seeks BC/BE Obstetrician/Gynecologist to join 8 physician Ob/Gyn department. Single site, single hospital practice. Competitive salary and fringe benefits.

Forward Inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI
53052-0427

OBSTETRICIAN/ GYNECOLOGIST

The Department of OB/GYN at The Mary Imogene Bassett Hospital is seeking a fifth full-time salaried attending obstetrician/gynecologist. The Bassett Hospital, teaching affiliate of Columbia University, is a multispecialty group practice which provides primary and tertiary care services to a 10 county predominantly rural region of upstate New York. Special features of this professional opportunity include a collaborative practice with four certified nurse-midwives, in-hospital birthing center, laparoscopic surgery, laser surgery, infertility and a Gyn Oncologist on the consulting staff. Cooperstown, a unique lakeside village, enjoys recreational facilities, excellent schools and a wide range of cultural activities. We offer a competitive salary, faculty appointment, and a liberal fringe benefits, including full malpractice coverage. Please send CV and correspondence to:

Marc E. Heller, M.D.,
Chief Department of OB/GYN
The Mary Imogene Bassett Hospital
1 Atwell Road, Cooperstown, NY 13326
(607) 547-3170

SOLO OR PARTNERSHIP OPPORTUNITY MITCHELL, SOUTH DAKOTA

Two OB/GYN's are currently serving draw area of 40,000. Local, 120-bed hospital provides excellent facilities. Tertiary services are accessible in Sioux Falls. You'll have full support of this quality medical staff. Mitchell is an attractive community and has excellent schools, a private university, a strong economy, and low cost of living. Area offers plenty of recreational amenities, including water sports, hunting, golf, and fishing. Competitive income package offered and earning potential is well above average. Send CV or call:

Bryan Bassett
JACKSON AND
COKER, INC.
115 Perimeter
Center Place
Suite 380 10764
Atlanta, GA 30346
Tel. 1-800-544-1987

JACKSON AND COKER

OBSTETRICS & GYNECOLOGY

Physicians, Midwives, Physician's Assistants

Mount Sinai School of Medicine

Major teaching affiliation minutes from midtown Manhattan has excellent opportunities for Board certified/eligible Obstetricians/Gynecologists, Certified Nurse-Midwives, and NYS-licensed Physician's Assistants at Elmhurst Hospital Center serving western Queens community. Full/part-time opportunities available in all areas including day and evening clinics. Join our team providing OB/GYN healthcare to challenging patient mix in culturally diverse environment.

Excellent salary and benefits package for qualified candidates. Physicians will be granted academic rank based upon individual credentials. Participation available in successful physician private practice plan. Send c.v. or call: **Jasmin Moshirpur, MD, Director of Obstetrics & Gynecology/Associate Dean for Elmhurst Affairs, Room #C8-13, (718) 830-1340/1348.**

MOUNT SINAI SCHOOL OF MEDICINE OF THE CITY UNIVERSITY OF NEW YORK

MOUNT SINAI SERVICES
Elmhurst Hospital Center
79-01 Broadway, Elmhurst, NY 11373
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Maternal Fetal Medicine Subspecialist

Faculty Positions

The James H. Quillen College of Medicine of East Tennessee State University (ETSU) is seeking board certified/eligible Maternal-Fetal Medicine (Perinatology) subspecialists. These positions offer an excellent opportunity combining research, teaching and private practice with a faculty position at the Assistant/Associate Professor level. Salaries are very competitive and fringe benefits are excellent. The Medical School is located in the Tri-Cities area of Northeast Tennessee in a beautiful section of the Appalachians and within seeing distance of the Blue Ridge Mountains. The area has excellent hunting, fishing, and camping with multiple ski resorts at your doorstep. The Tri-Cities combine good schools and rural living in a safe, beautiful environment. Yet, the medical referral base consists of 1.3 million people within a 50 mile radius of the Medical School. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman, Dept. OB/GYN
James H. Quillen College of Medicine
East Tennessee State University
P.O. Box 19570A
Johnson City, TN 37614-0002

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The **Michael Reese Health Plan,** a large well-established HMO serving its members in 20 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in

Obstetrics Gynecology

Physicians with the Health Plan enjoy a full practice with modern facilities and equipment as well as opportunities for teaching. All specialty facilities available.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Alfred Kendrick, M.D.
Medical Affairs
MICHAEL REESE HEALTH PLAN
2545 King Drive, Chicago 60616
or call (312) 808-4551

Michael Reese Health Plan

an equal opportunity employer m/f/h

EUGENE, OREGON

BC/BE obstetrician/gynecologist to join two-physician obstetric/gynecology department in a 44-physician multi-specialty group. Excellent schools. Abundant cultural and recreational opportunities. Near Cascade Mountains and the scenic coast. Home of University of Oregon. Level III nursery with perinatologist, excellent salary and benefits package, including malpractice insurance, liberal leave and two pension plans leading to partnership. Please send CV to:

David Powell, MD
Oregon Medical Group
175 West B, Suite I
Springfield, OR 97477
or phone
(503) 726-1010

STAFF PERINATOLOGIST

Our **Maternal Fetal Medicine Section** is seeking to increase its current staff of six perinatologists and one geneticist, to cover our rapidly expanding responsibilities in the fields of prenatal diagnosis and high risk obstetrics.

PENNSYLVANIA HOSPITAL, the Nation's First Hospital, is a tertiary care center, located in the historic Society Hill Section of Philadelphia.

Our section provides perinatal consultation, ultrasound evaluation, and fetal testing to an extensive referral network of surrounding institutions. Pennsylvania Hospital is also the site of the International PUBS Registry, has an active prenatal diagnosis program involving CVS and amniocentesis, and a committment to teaching with four Maternal Fetal Medicine Fellows.

Faculty appointment at the **University of Pennsylvania School of Medicine** is available. Malpractice insurance coverage and a liberal benefit package are offered. All applicants must be board certified or board eligible.

For more information, please write or contact:

Stuart Weiner, M.D.
Section Chief, Maternal Fetal Medicine
Department of Obstetrics/Gynecology
Pennsylvania Hospital
800 Spruce Street
Philadelphia, PA 19107
(215) 829-5808

Pennsylvania Hospital is an affirmative equal/action opportunity employer.

PERINATAL ASSOCIATES OF NORTHERN CALIFORNIA

Are you a clinical perinatologist? There is still time to join four perinatologists and one geneticist-OB/GYN looking for one more perinatologist who: desires superior environment for patient care, in a private, modern, level 3 facility doing 6,000 deliveries a year with superior perinatal outcome; likes personal, direct hands-on patient care; needs PUBS, IUTxF, CVS and complicated pregnancies, level 2 ultrasound; demands partners, not superiors; wants to live in clean, warm, not humid, snow-free (but 60 minutes from skiing) Sacramento, California; feels one's family has priorities, and is committed to the private practice of medicine. For more information, call (916) 733-1755, extension 2773.

Hanns C. Haesslein, M.D.
Perinatal Associates
of Northern California
5301 F Street
Sacramento, California 95819

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

Kaiser Permanente offers competitive salaries and excellent benefits including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE

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Good People. Good Medicine.

OB/GYN PHYSICIAN

Established, high quality practice in upstate NY is expanding its facilities. Currently with five OB/GYN (BC/BE) physicians, two certified nurse midwives, and two nurse practitioners. Services include high-risk OB; infertility, genetic, nutrition and psychological counseling; mammography; ultrasound; laser and micro-surgery.

Lucrative compensation program and partnership opportunity. Complete benefit/ insurance package. Limited call schedule; dynamic quality of life.

Please contact:
Sharon Perkins, Box A,
Associates in Women's Medical Health Care,
2123 River Road, Schenectady, NY 12309.
Or call (518) 381-1121.

Finger Lakes Region
Ithaca, New York

Unique opportunity for patient oriented physician in comprehensive OB-GYN service, building on established patient base of 6,000 patients. To include out-patient gynecology, obstetrics, surgery, infertility, and abortions. First call protection from nurse-midwives. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Summer/Fall 1991.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

Planned Parenthood®
of Tompkins County

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for four (4) full-time faculty positions for a new women's health center and academic program at its Milwaukee Clinical Campus.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5500 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin and St. Luke's Medical Center in Milwaukee.

Qualifications include board candidacy or certification in obstetrics and gynecology and a commitment to teaching residents, medical students, and clinical research. Qualifications for the four positions for GENERAL OB/GYN can take into consideration special individual interests. Special expertise of interest for the department are:

- STATEWIDE FAMILY PLANNING AND AMBULATORY CARE (Part-time medical director, Planned Parenthood)
- OPERATIVE LAPAROSCOPY, HYSTEROSCOPY
- UROGYNECOLOGY
- RESIDENCY/MEDICAL STUDENT EDUCATION
- OB/GYN ULTRASOUND

Private practice opportunities are also available in an associated offsite multispecialty clinic and all faculty members participate in a group practice at the institution. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/
Gynecology
University of Wisconsin
Medical School
600 Highland Avenue, H4/564 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/
Gynecology
University of Wisconsin
Medical School
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

The University of Wisconsin Medical School
is an equal opportunity employer.

UCLA INTERCAMPUS MEDICAL GENETICS TRAINING PROGRAM

The UCLA intercampus post-doctoral research training program in Medical Genetics utilizes the research and clinical resources of its affiliated campuses and teaching hospitals. The program is open to academically oriented applicants with the M.D., Ph.D., D.D.S. or equivalent degrees. A wide variety of research training opportunities in molecular, biochemical, immuno-, cancer, cyto-, somatic cell, population and clinical genetics are available. The program also meets all the requirements of the American Board of Medical Genetics. Clinical genetics fellowships are available at each of the affiliated hospitals. Application forms are available from David Rimoin, M.D., Ph.D., Department of Pediatrics, Cedars-Sinai Medical Center, 8700 Beverly Blvd., Los Angeles, CA 90048.

MATERNAL-FETAL MEDICINE

The University of North Carolina at Chapel Hill Department of Obstetrics and Gynecology in conjunction with the Wake Medical Center, Raleigh, North Carolina, and the Wake Area Health Education Center has an opening for a board certified or active candidate perinatologist with a special interest in ultrasound and genetics. The position is in the Wake AHEC OB/GYN Department which has two full-time obstetricians and gynecologists, plus the program director who is board certified in Maternal-Fetal Medicine.

Wake Medical Center is located in Raleigh, approximately 40 minutes from Chapel Hill, and is a 560-bed comprehensive tertiary care center with over 4000 deliveries per year and a neonatal intensive care unit staffed by four neonatologists.

Excellent opportunity for research and teaching of residents and medical students rotating through Wake Medical Center at all times. Collaboration with the Maternal-Fetal Medicine Division at the University of North Carolina is encouraged and anticipated.

Academic appointment and salary dependent on training and experience.

Write or contact:

Juan L. Granados, M.D.
OB/GYN Wake AHEC Director
Wake County Medical Center
3000 New Bern Avenue
Raleigh, NC 27610
(919) 250-8535

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FACULTY POSITION
DIVISION DIRECTOR
MATERNAL-FETAL MEDICINE

- ANTENATAL UNIT
- 5500 DELIVERIES/YEAR
- PREMIER NEWBORN INTENSIVE CARE UNIT
- 7 NEONATOLOGISTS
- 24 HR. IN HOUSE OBSTETRICAL ANESTHESIA
- ULTRASOUND LAB (2 - ACUSON 128XP)
- INTERVENTIONAL SONOGRAPHIC EXPERIENCE
- AIR/GROUND TRANSPORT SYSTEM
- LARGE REFERRAL AREA
- LOW COST OF LIVING
- EXTREMELY COMPETITIVE COMPENSATION PACKAGE
- RESEARCH/GRANT OPPORTUNITIES
- TEACHING RESIDENTS & STUDENTS

The Department of Obstetrics & Gynecology at the University of Kansas School of Medicine-Wichita is accepting applications for the position of Director of the Division of Maternal-Fetal Medicine. This represents a unique opportunity to have the best features of both academic medicine and private practice.

Please send curriculum vitae to:

Daniel K. Roberts, M.D., Ph.D.
Professor & Chairman, Dept. of Ob/Gyn
University of Kansas School of Medicine-Wichita
HCA-Wesley Medical Center
550 N. Hillside
Wichita, KS 67214

An Affirmative Action/Equal Opportunity Employer

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting for positions in our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

REPRODUCTIVE ENDOCRINOLOGIST

• SAN JOSE •

We are currently seeking a Reproductive Endocrinologist to establish and run a reproductive endocrinology subdivision in the department. Duties would include the full spectrum of reproductive endocrinology, outpatient and surgical management of infertility. Fellowship certification would be preferred. The department currently has 17 physicians and is still growing. We offer a generous benefits package including full coverage malpractice insurance. San Jose has beautiful weather and excellent access to Bay Area recreational and cultural activities.

For more information, please contact: Jerome L. Nehemiah, M.D., Chief, Department of OB/GYN, The Permanente Medical Group, Inc., 260 International Circle, Dept. AJOBG, San Jose, CA 95119. or call collect (408) 972-6180. EOE

KAISER PERMANENTE

Good People. Good Medicine.

FACULTY POSITIONS IMMEDIATELY AVAILABLE

Maternal Fetal Medicine — Reproductive Endocrinology — Obstetrics/Gynecology — Urogynecology

The Department of Obstetrics and Gynecology at the University of South Dakota School of Medicine in Sioux Falls, South Dakota, a delightful city located directly on the Sioux River and serving a tri-state area and one of the best kept secrets in the Midwest, is building a full-time academic department. Qualified individuals to contribute, develop and provide leadership in all aspects of the specialty are actively being sought.

Candidates should have interests in clinical practice activities and medical education. Clinical and/or basic research opportunities are available and encouraged. An opportunity for a creative, motivated individual with a pioneer spirit.

Financial remuneration and incentives are competitive and commensurate with experience, credentials and rank. BC or BE. Position open until filled.

Please send Curriculum Vitae to: R. H. Wiebe, M.D., Professor and Chairman, Department of Obstetrics and Gynecology, University of South Dakota School of Medicine, 1100 South Euclid Avenue, Sioux Falls, SD 57117-5039, (605) 333-7327.

An Equal Opportunity/Affirmative Action Employer

OBSTETRICIAN & GYNECOLOGIST

Wyeth-Ayerst Laboratories, a leading multinational pharmaceutical/nutritional manufacturer responsible for the development of female health products, has an immediate position available in its Radnor, PA headquarters for a board certified Obstetrician and Gynecologist.

A minimum of 5 years private practice and some experience in clinical research is desired. The successful candidate will assist in the development and implementation of clinical protocols and case report forms, monitor clinical studies, interpret data and assist in medical marketing liaisons. Good communicative skills and a willingness to travel worldwide are required.

We offer competitive salaries and compensation programs in a modern, professional setting. For immediate consideration, forward your resume with salary requirements to: **Wyeth-Ayerst Laboratories, Position #172, P.O. Box 8299, Philadelphia, PA 19101.** An equal opportunity employer, M/F/H/V responses encouraged. Principals Only.

WYETH
AYERST
™

Head—Department of Obstetrics and Gynecology

Applications are being solicited for the position of Head and Tenured Professor of the Department of Obstetrics and Gynecology, University of Minnesota Medical School and Chief of Service of Obstetrics and Gynecology of the University of Minnesota Hospital and Clinic, Minneapolis, Minnesota. We seek a candidate with outstanding academic credentials who is Board certified in Obstetrics and Gynecology with demonstrated experience and skill to provide leadership in administration, research, service and teaching of medical, graduate and allied health students in the health sciences of a large multi-faceted department. The candidate must also have a national reputation in his/her professional field. Submit inquiries and curriculum vitae by July 31, 1991 to:

George L. Adams, M.D.
Chair, Ob-Gyn Search Committee
University of Minnesota Medical School
Box 396 UMHC
420 Delawre Street S.E.
Minneapolis, Minnesota 55455

The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities.

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

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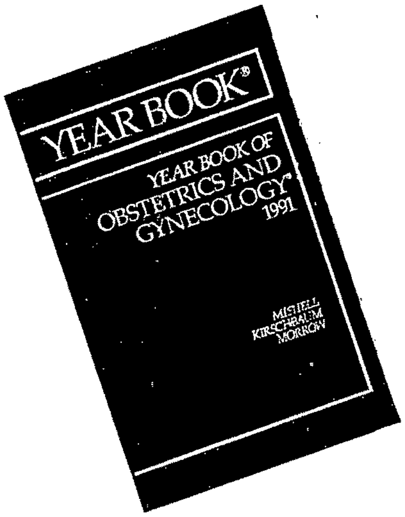

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7. Did you know that if you use cocaine, are addicted to heroin, or are methadone-dependent, your children will be at a greater risk for sudden infant death syndrome (SIDS)? ☐ YES ☐ NO

8. Did you know that if you use barbiturates during your pregnancy you can change your child's normal patterns of growth and behavior? Your child might also suffer withdrawal symptoms for months after birth, including high-pitched crying, irritability, and sleep disturbances. ☐ YES ☐ NO

9. Is your doctor aware of all the prescription and/or illicit drugs you may be taking? ☐ YES ☐ NO

Did you know that if you have a drug problem, there are many people and resources you can turn to for help? Just ask your doctor.

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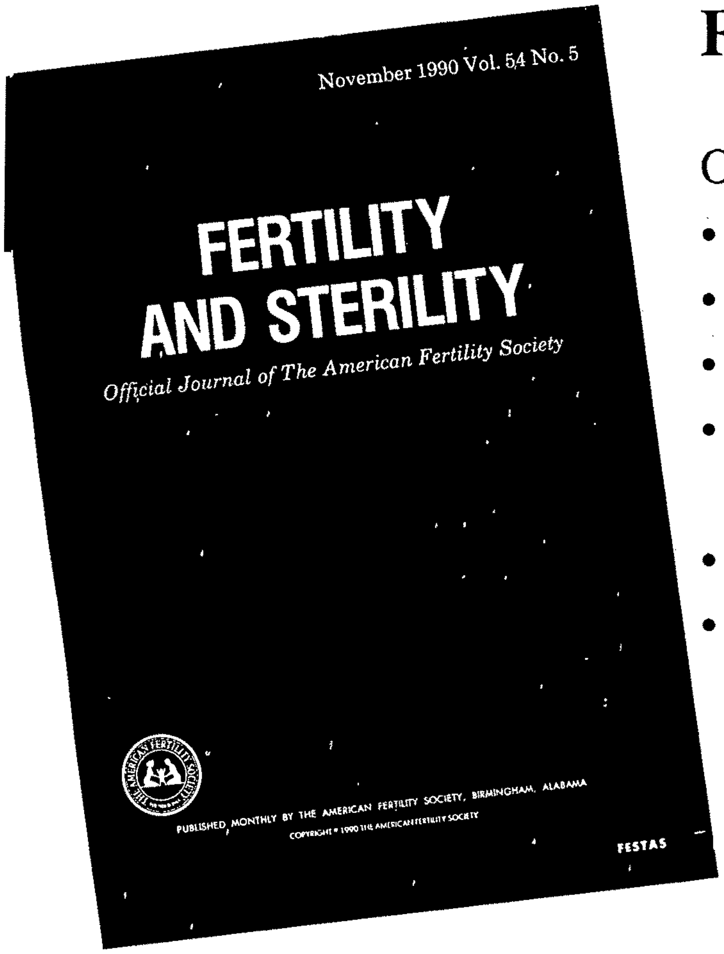

November 1990 Vol. 54 No. 5

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FERTILITY AND STERILITY

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MPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

NDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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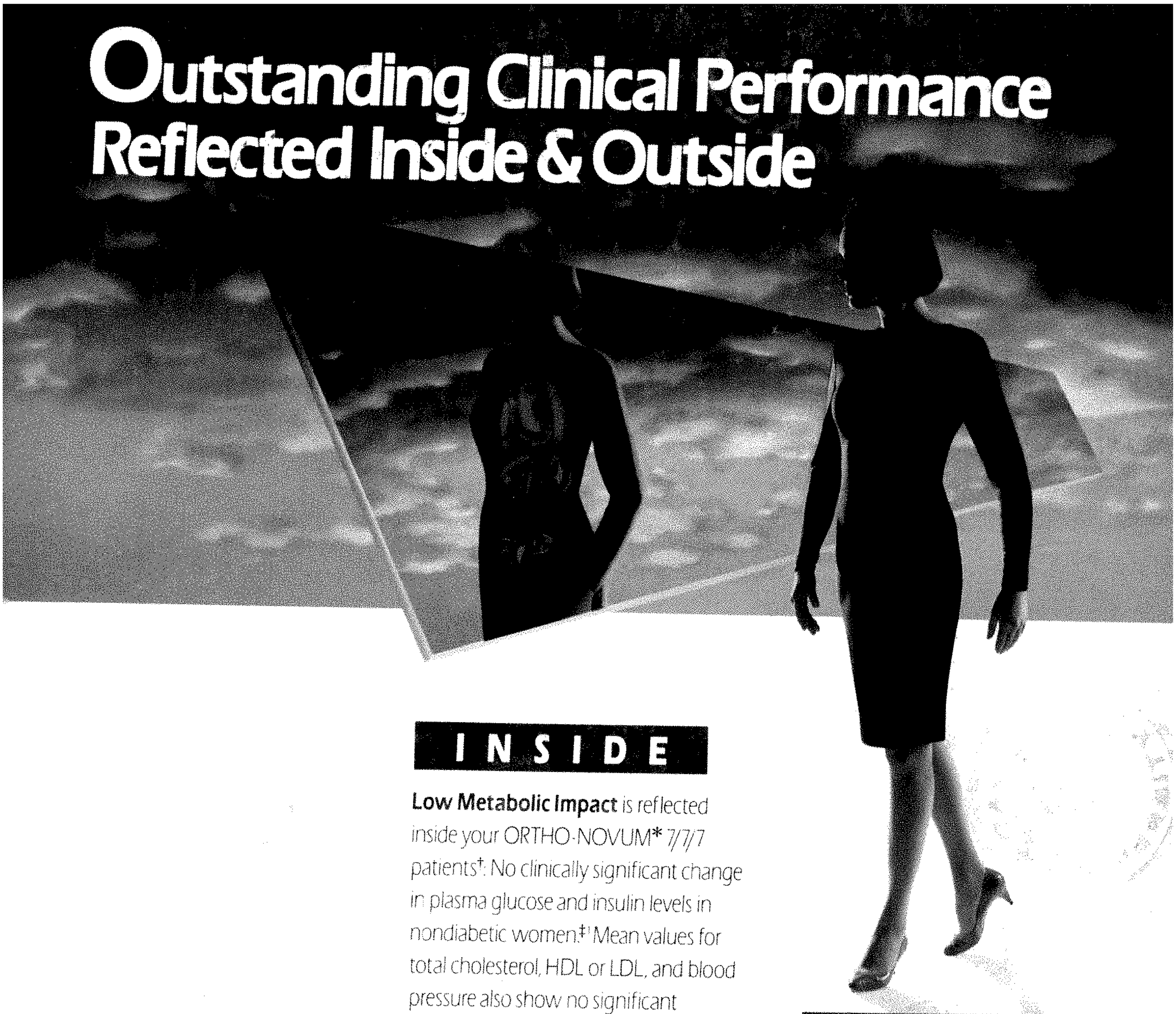

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[*] Trademark

[†] Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

[‡] Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

[§] An increase in blood pressure has been reported in women taking oral contraceptives.

[1] Spellacy WN, Ellingson AB, Tsibris JCM: Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med* 1989;34(8):540-542.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

ORTHO PHARMACEUTICAL
CORPORATION
Raritan, New Jersey 08869

ORTHO

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OJ-988

TRI-NORINYL®

Uniquely phased for all phases of a woman's reproductive life

ORAL CONTRACEPTIVE AGENTS: BRIEF SUMMARY

DESCRIPTION

TRI-NORINYL 21-DAY Tablets provide an oral contraceptive regimen of 7 blue tablets followed by 9 yellow-green tablets and 5 more blue tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg and each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg.

TRI-NORINYL 28-DAY Tablets provide a continuous oral contraceptive regimen of 7 blue tablets, 9 yellow-green tablets, 5 more blue tablets, and then 7 orange tablets. Each blue tablet contains norethindrone 0.5 mg and ethinyl estradiol 0.035 mg, each yellow-green tablet contains norethindrone 1 mg and ethinyl estradiol 0.035 mg, and each orange tablet contains inert ingredients.

INDICATIONS AND USAGE

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use these products as a method of contraception.

CONTRAINDICATIONS

Oral contraceptives should not be used in women who have the following conditions: Thrombophlebitis or thromboembolic disorders • A past history of deep vein thrombophlebitis or thromboembolic disorders • Cerebral vascular or coronary artery disease • Known or suspected carcinoma of the breast • Carcinoma of the endometrium, and known or suspected estrogen-dependent neoplasia • Undiagnosed abnormal genital bleeding • Cholestatic jaundice of pregnancy or jaundice with prior pill use • Hepatic adenomas, carcinomas or benign liver tumors • Known or suspected pregnancy

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia and gallbladder disease, although the risk of serious morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, hypercholesterolemia, obesity and diabetes.

Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with formulations containing 0.05 mg or higher of estrogen. The effects of long-term use with lower dose formulations of both estrogens and progestogens remain to be determined.

Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease. Relative risk, the *ratio* of the incidence of a disease among oral contraceptive users to that among non-users, cannot be assessed directly from case control studies, but the odds ratio obtained is a measure of relative risk. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide not only a measure of the relative risk but a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and non-users. The attributable risk does provide information about the actual occurrence of a disease in the population.

1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS

a. Myocardial Infarction

An increased risk of myocardial infarction has been attributed to oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be 2 to 6. The risk is very low under the age of 30. However, there is the possibility of a risk of cardiovascular disease even in very young women who take oral contraceptives.

Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women 35 or older, with smoking accounting for the majority of excess cases.

Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives.

Oral contraceptives may compound the effects of well-known risk factors for coronary artery disease, such as hypertension, diabetes, hyperlipidemias, hypercholesterolemia, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and impair oral glucose tolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see **WARNINGS**, section 9). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. Thromboembolism

An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. One cohort study has shown the relative risk to be somewhat lower, about 3 for new cases (subjects with no past history of venous thrombosis or varicose veins) and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped.

A 2- to 6-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. If feasible, oral contraceptives should be discontinued at least 4 weeks prior to and for 2 weeks after elective surgery and during and following prolonged immobilization. Since the immediate postpartum period also is associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than 4 to 6 weeks after delivery in women who elect not to breast feed.

c. Cerebrovascular diseases

An increase in both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes) has been shown in users of oral contraceptives. In general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users for both types of strokes while smoking interacted to increase the risk for hemorrhagic strokes.

In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk also is greater in women 35 or older and among smokers.

d. Dose-related risk of vascular disease from oral contraceptives

A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with some progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogens used in the contraceptives. The amount of both hormones should be considered in the choice of an oral contraceptive.

Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing the lowest estrogen content that produces satisfactory results for the individual.

e. Persistence of risk of vascular disease

There are three studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for 5 or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. Subarachnoid hemorrhage also has a significantly increased relative risk after termination of use of oral contraceptives. However, these studies were performed with oral contraceptive formulations containing 0.05 mg or higher of estrogen.

2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE

One study gathered data from a variety of sources which have estimated the mortality rates associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral contraceptive users is based on data gathered in the 1970's—but not reported in the U.S. until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral contraceptive use to women who do not have the various risk factors listed in this labeling.

Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular disease risks may be increased with oral contraceptive use after age 40 in healthy non-smoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception.

Therefore, the Committee recommended that the benefits of oral contraceptive use by healthy non-smoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. CARCINOMA OF THE BREAST AND REPRODUCTIVE ORGANS

Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and non-users and differences in age at start of use, has been questioned. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use.

Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors.

In spite of many studies of the relationship between oral contraceptive use and breast or cervical cancers, a cause and effect relationship has not been established.

4. HEPATIC NEOPLASIA

Benign hepatic adenomas are associated with oral contraceptive use although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases per 100,000 for users, a risk that increases after 4 or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage.

Studies in the United States and Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (> 8 years) oral contraceptive users. However, these cancers are extremely rare in the United States and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users is less than 1 per 1,000,000 users.

5. OCULAR LESIONS

There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY

Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. More recent studies do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy.

The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion.

It is recommended that for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued if pregnancy is confirmed.

7. GALLBLADDER DISEASE

Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS

Oral contraceptives have been shown to impair oral glucose tolerance. Oral contraceptives containing greater than 0.075 mg of estrogen cause glucose intolerance with impaired insulin secretion, while lower doses of estrogen may produce less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives.

Some women may develop persistent hypertriglyceridemia while on the pill. As discussed earlier (see **WARNINGS**, sections 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users.

9. ELEVATED BLOOD PRESSURE

An increase in blood pressure has been reported in women taking oral contraceptives. The incidence of risk also was reported to increase with continued use and among older women. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing concentrations of progestogens.

Women with a history of hypertension or hypertension-related diseases or renal disease should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives and there is no difference in the occurrence of hypertension among ever- and never-users.

10. HEADACHE

The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. BLEEDING IRREGULARITIES

Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first 3 months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out.

Some women may encounter post-pill amenorrhea or oligomenorrhea, especiall when such a condition was pre-existent.

PRECAUTIONS

1. PHYSICAL EXAMINATION AND FOLLOW-UP

A complete medical history and physical examination should be taken prior to the ini ation or reinstitution of oral contraceptives and at least annually during use of oral co traceptives. These physical examinations should include special reference to bloo pressure, breasts, abdomen and pelvic organs, including cervical cytology and rel vant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vagina bleeding, appropriate diagnostic measures should be conducted to rule out mali nancy. Women with a strong family history of breast cancer or who have breast nodule should be monitored with particular care.

2. LIPID DISORDERS

Women who are being treated for hyperlipidemias should be followed closely if the elect to use oral contraceptives. Some progestogens may elevate LDL levels and ma render the control of hyperlipidemias more difficult.

3. LIVER FUNCTION

If jaundice develops in any woman receiving oral contraceptives the medication shoul be discontinued. Steroid hormones may be poorly metabolized in patients wit impaired liver function.

4. FLUID RETENTION

Oral contraceptives may cause some degree of fluid retention. They should be pre scribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention.

5. EMOTIONAL DISORDERS

Women with a history of depression should be carefully observed and the drug discon tinued if depression recurs to a serious degree.

6. CONTACT LENSES

Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist.

7. DRUG INTERACTIONS

Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar associ ation though less marked, has been suggested with barbiturates phenylbutazone phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines.

8. INTERACTIONS WITH LABORATORY TESTS

Certain endocrine and liver function tests and blood components may be affected by oral contraceptives:

a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.

b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG. Free T4 concentration is unaltered.

c. Other binding proteins may be elevated in serum.

d. Sex steroid binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged.

e. Triglycerides may be increased.

f. Glucose tolerance may be decreased.

g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives.

9. CARCINOGENESIS

See **WARNINGS** section.

10. PREGNANCY

Pregnancy Category X. See **CONTRAINDICATIONS** and **WARNINGS** sections.

11. NURSING MOTHERS

Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

INFORMATION FOR THE PATIENT

See **PATIENT LABELING.**

ADVERSE REACTIONS

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see **WARNINGS** section): Thrombophlebitis • Arterial thromboembolism • Pulmonary embolism • Myocardial infarction • Cerebral hemorrhage • Cerebral thrombosis • Hypertension • Gallbladder disease • Hepatic adenomas, carcinomas or benign liver tumors

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: Mesenteric thrombosis • Retinal thrombosis

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea • Vomiting • Gastrointestinal symptoms (such as abdominal cramps and bloating) • Breakthrough bleeding • Spotting • Change in menstrual flow • Amenorrhea • Temporary infertility after discontinuation of treatment • Edema • Melasma which may persist • Breast changes: tenderness, enlargement, secretion • Change in weight (increase or decrease) • Change in cervical erosion and secretion • Diminution in lactation when given immediately postpartum • Cholestatic jaundice • Migraine • Rash (allergic) • Mental depression • Reduced tolerance to carbohydrates • Vaginal candidiasis • Change in corneal curvature (steepening) • Intolerance to contact lenses

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome • Cataracts • Changes in appetite • Cystitis-like syndrome • Headache • Nervousness • Dizziness • Hirsutism • Loss of scalp hair • Erythema multiforme • Erythema nodosum • Hemorrhagic eruption • Vaginitis • Porphyria • Impaired renal function • Hemolytic uremic syndrome • Budd-Chiari syndrome • Acne • Changes in libido • Colitis

OVERDOSAGE

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

NON-CONTRACEPTIVE HEALTH BENEFITS

The following non-contraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral contraceptive formulations containing estrogen doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: • Increased menstrual cycle regularity • Decreased blood loss and decreased incidence of iron deficiency anemia • Decreased incidence of dysmenorrhea

Effects related to inhibition of ovulation: • Decreased incidence of functional ovarian cysts • Decreased incidence of ectopic pregnancies

Effects from long-term use: • Decreased incidence of fibroadenomas and fibrocystic disease of the breast • Decreased incidence of acute pelvic inflammatory disease • Decreased incidence of endometrial cancer • Decreased incidence of ovarian cancer

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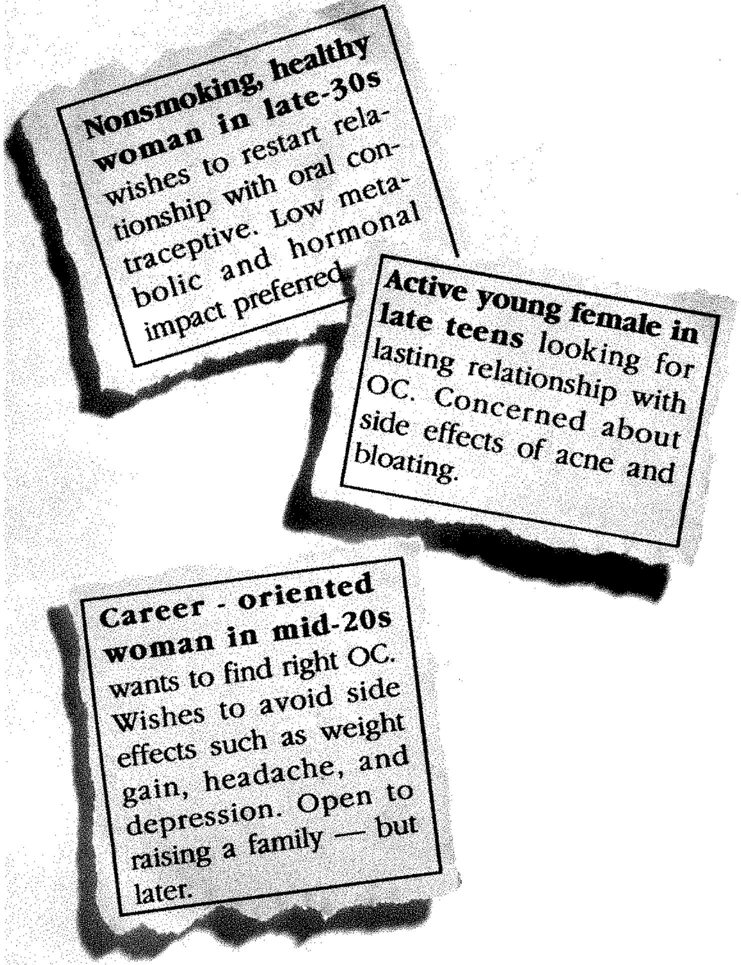
814-H2-170-90

Rev. May 1990

SYNTEX

SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304



Nonsmoking, healthy woman in late-30s wishes to restart relationship with oral contraceptive. Low metabolic and hormonal impact preferred.

Active young female in late teens looking for lasting relationship with OC. Concerned about side effects of acne and bloating.

Career - oriented woman in mid-20s wants to find right OC. Wishes to avoid side effects such as weight gain, headache, and depression. Open to raising a family — but later.

A personalized approach to oral contraceptives.

Because Tri-Norinyl is uniquely phased for all phases of a woman's reproductive life, it's ideal for a wide range of patient types:

Older, nonsmoking patients restarting contraception: The only multiphasic with norethindrone that *lowers* progestin in late cycle to minimize overall hormonal and metabolic impact.

Career-oriented patients delaying childbearing: Reduced progestin helps minimize side effects such as weight gain, headache, and depression.

Younger patients just starting contraception: Low-impact progestin lowers risk of acne and bloating; mid-cycle increase enhances support when breakthrough bleeding is most likely to occur.

(NORETHINDRONE AND ETHINYL ESTRADIOL) TABLETS

TRI-NORINYL®

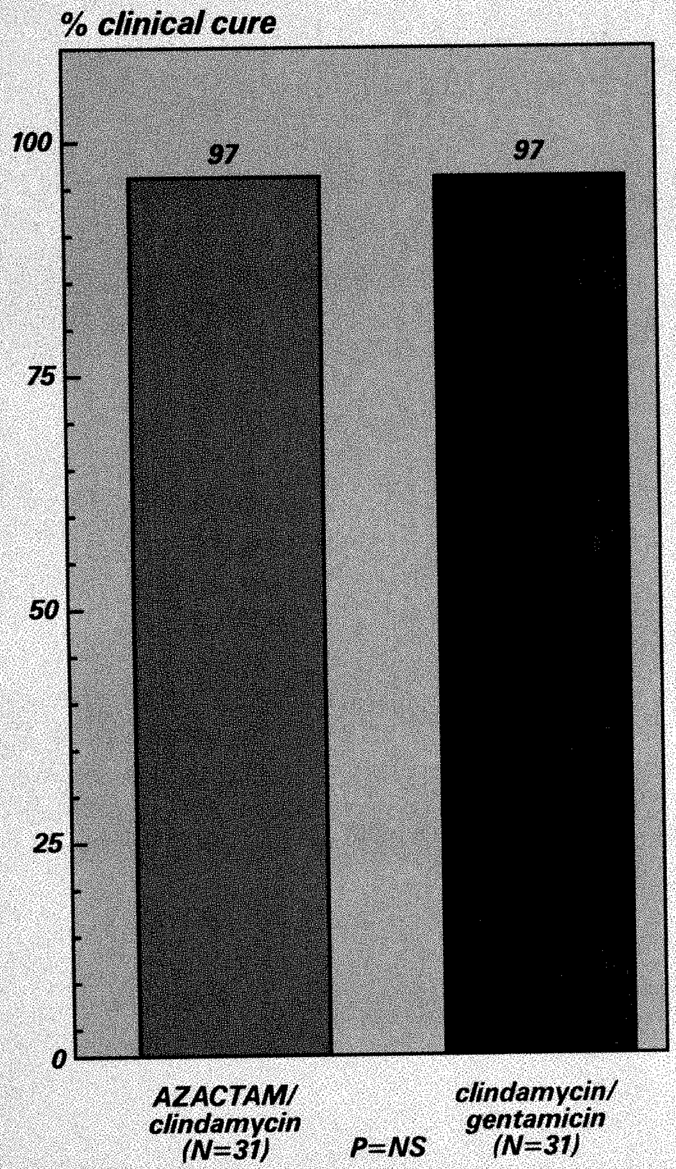
Uniquely phased for all phases of a woman's reproductive life

Committed to the OB/GYN and women's healthcare

SYNTEX
SYNTEX LABORATORIES, INC.
PALO ALTO, CA 94304

Serious as well as minor side effects have been reported following the use of all oral contraceptives. These include thromboembolic disease. Please see next page for brief summary of prescribing information. © 1990 Syntex Laboratories, Inc. 814-H2-201-90

IN POSTPARTUM ENDOMETRITIS*



A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

No universal criteria exist that may satisfy a single definition of "cure." Use of this term may vary between clinical settings and investigators, and the reader is urged to consult the reference listed.

Azactam®

aztreonam

delivers...

---

*Due to susceptible organisms.

†Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

‡Antibiotics should be given with caution to any patient who has had some forr of allergy, particularly to drugs. See full prescribing information for recommer dations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions oc curred in less than 2% of patients, including local reactions, diarrhea, nause vomiting, and rash.

more than you're expecting...

- *Broad aerobic gram-negative in vitro coverage, including Escherichia coli, Proteus mirabilis, and Enterobacter cloacae.*[†]
- *Greater than 90% cure rates in combination with clindamycin.*[1-3]
- *No time-consuming, costly, routine therapeutic drug monitoring.*
- *May be admixed in the same bag with clindamycin.*
- *May be used in penicillin-allergic patients.*[‡]
- *Pregnancy Category B.*

There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.

Azactam®

aztreonam

A logical alternative to aminoglycosides.

Please see adjacent page for brief summary of prescribing information.

Azactam®

aztreonam

AZACTAM* FOR INJECTION
Aztreonam For Injection

DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylacti or urticarial) to penicillins and/or cephalosporins should be followed with special care If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or i therapy is prolonged, renal function should be monitored because of the potentia nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

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*Efficacy for this organism in this organ system was studied in fewer than ten infections.

***References:* 1.** *Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* **2.** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* **3.** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

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American Journal of Obstetrics and Gynecology

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July
1991

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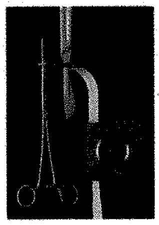

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CEFOTAN® IM/IV
(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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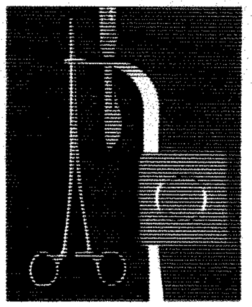

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In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
BRIEF SUMMARY

INDICATIONS AND USAGE

TREATMENT: CEFOTAN® (cefotetan disodium) is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary tract infections caused by *E coli, Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis* and *Proteus* sp (which may include the organisms now called *Proteus vulgaris, Providencia rettgeri,* and *Morganella morganii*).

Lower respiratory tract infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), and *E coli.*

Skin and skin structure infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus pyogenes* and *Streptococcus* species (excluding enterococci), and *E coli.*

Gynecologic infections caused by *Staphylococcus aureus** (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis, Streptococcus* species (excluding enterococci), *E coli, Proteus mirabilis, Neisseria gonorrhoeae, Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species*).

Intra-abdominal infections caused by *E coli, Klebsiella* species (including *K pneumoniae**), *Streptococcus* species (excluding enterococci), and *Bacteroides* species (excluding *B distasonis, B ovatus, B thetaiotaomicron*).

Bone and joint infections caused by *Staphylococcus aureus.**

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

NOTE: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

PROPHYLAXIS: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis. Cholestyramine and colestipol resins have been shown to bind the toxin in vitro.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile.* Other causes should also be considered.

PRECAUTIONS

GENERAL: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity. Those at risk include patients with renal or hepatic impairment or poor nutritional state, the elderly and patients with cancer. Prothrombin times should be monitored in patients at risk and exogenous vitamin K administered as indicated.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

INFORMATION FOR PATIENTS: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

DRUG INTERACTIONS: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

DRUG/LABORATORY TEST INTERACTIONS: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be

CEFOTAN® (cefotetan disodium)

developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

USAGE IN PREGNANCY: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

USAGE IN NURSING MOTHERS: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

PEDIATRIC USE: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500). During postmarketing experience with CEFOTAN, anaphylactic reactions and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

TREATMENT: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

PROPHYLAXIS: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

IMPAIRED RENAL FUNCTION: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

HOW SUPPLIED

CEFOTAN is a dry, white to pale yellow powder supplied in vials containing cefotetan disodium equivalent to 1 g and 2 g cefotetan activity for intravenous and intramuscular administration. The 1 g dose is available in 10 mL and 100 mL vials, and the 2 g dose is available in 20 mL and 100 mL vials. CEFOTAN is also available in a pharmacy bulk package of 10 g in 100 mL vials. The vials should not be stored at temperatures above 22°C and should be protected from light.

1 g in 10 mL vial (NDC 0038-0376-10); 2 g in 20 mL vial (NDC 0038-0377-20); 1 g in 100 mL vial (NDC 0038-0376-11); 2 g in 100 mL vial (NDC 0038-0377-21); 10 g in 100 mL vial (NDC 0038-0375-10).

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To be continued...

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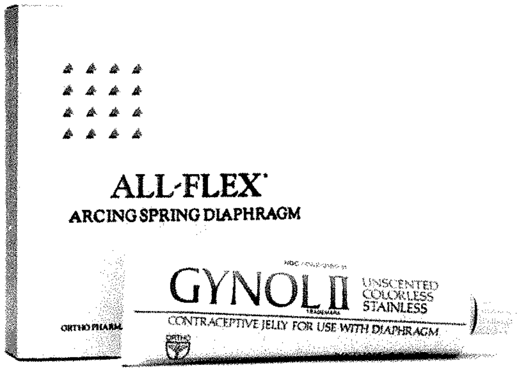

†Number of patients continuing the method for 12 consecutive months; data on file, Ortho Pharmaceutical Corporation.

‡Data on file, Ortho Pharmaceutical Corporation.

Consult the patient brochure for information on toxic shock syndrome (TSS) and wearing time.

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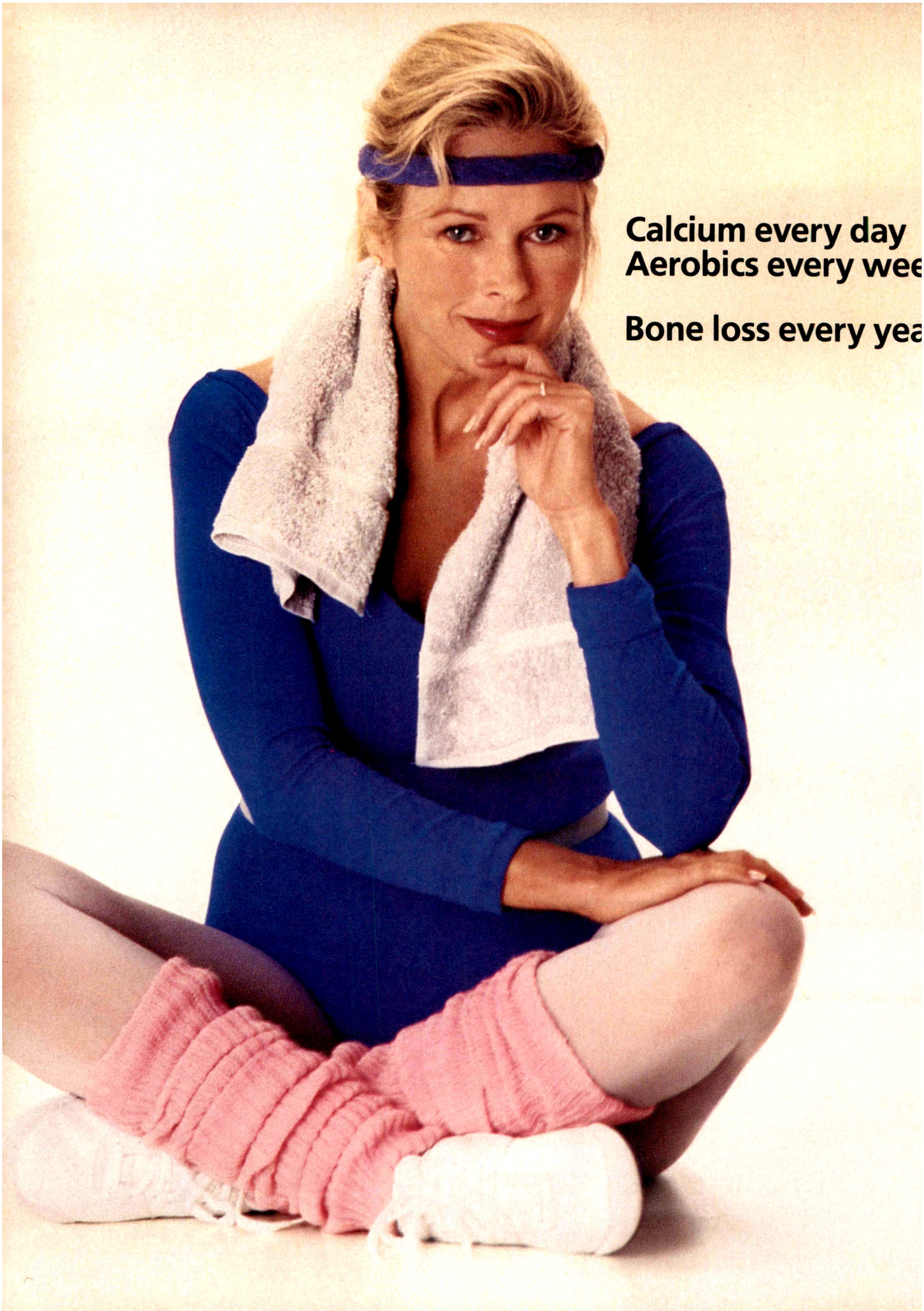
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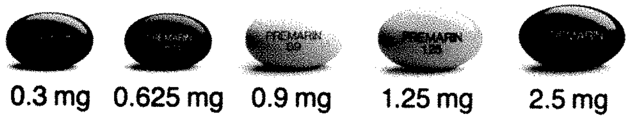

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BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.

There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one year without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develop manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding and mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should be used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associated with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progestin combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased, reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthetic estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver.

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATIONS** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be done only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleeding pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterine fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts; nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist when drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hair, hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, mental depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria; edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atrophic vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible. Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or taper medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 days followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule is repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downward according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effective control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued as promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should be monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event of persistent or recurring abnormal vaginal bleeding.

Revised August 21, 1989

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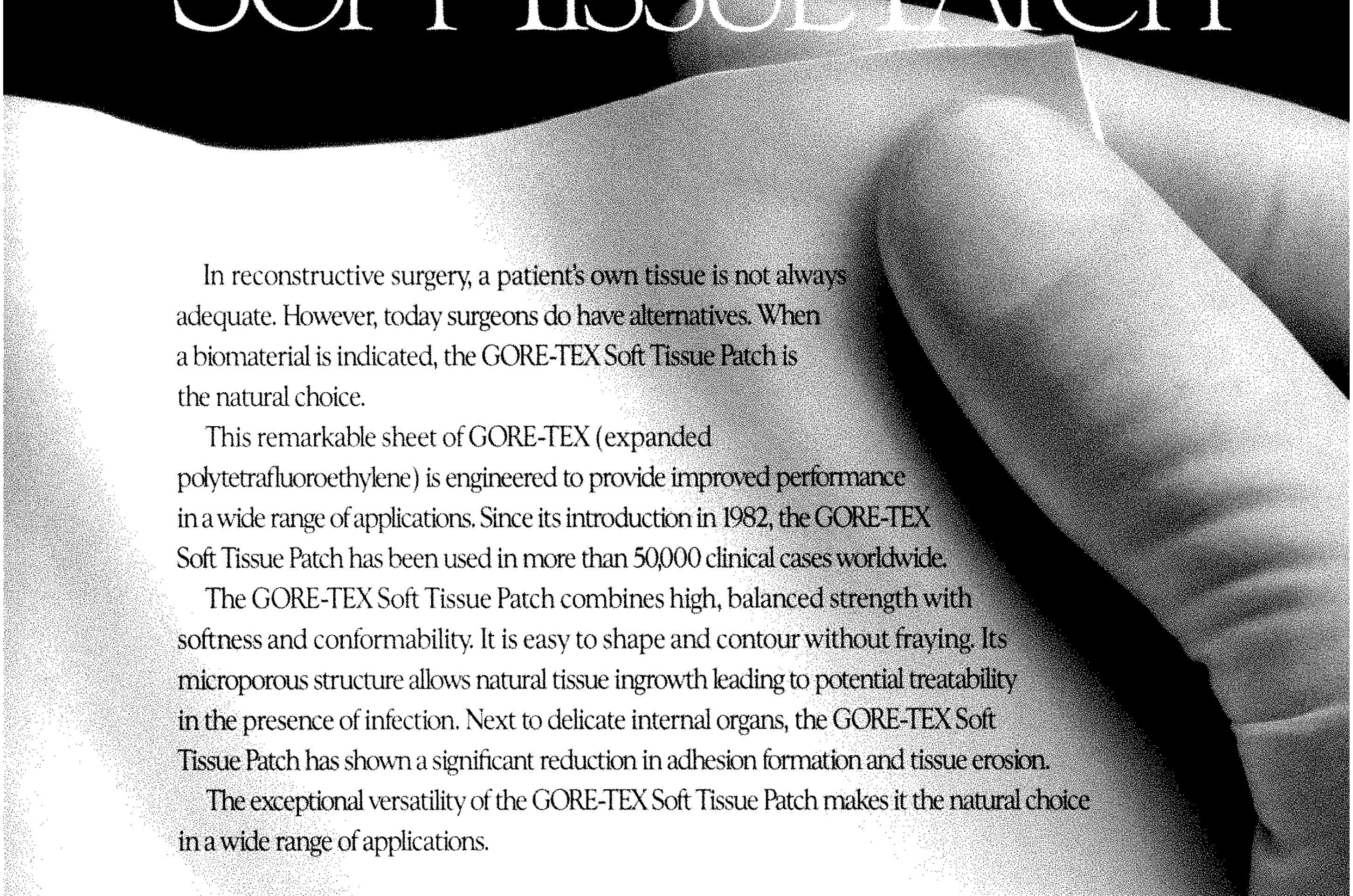

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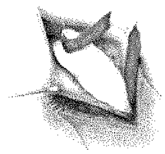

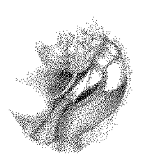
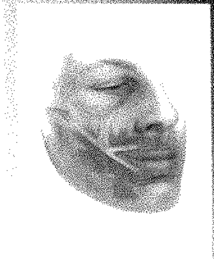

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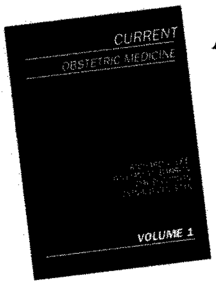

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Manuscript submission. Manuscripts should be submitted to one of the two Editors in Chief as follows:

1. **Dr. Zuspan**—manuscripts from the eastern United States and from England, Israel, and Italy, manuscripts written for Clinical Opinion and Current Development, Letters to the Editors, and all manuscripts presented before one of the official sponsoring societies, except the Society for Gynecologic Investigation. *Manuscripts from The Society of Perinatal Obstetricians should be submitted to Dr. Donald R. Coustan, Department of Obstetrics and Gynecology, Women and Infants Hospital of Rhode Island, 101 Dudley St., Providence, RI 02905.*
2. **Dr. Quilligan**—manuscripts from states west of Pennsylvania, West Virginia, Kentucky, Tennessee, and Mississippi; manuscripts from Hawaii, Alaska, Canada, and abroad (except England, Israel, and Italy). Dr. Quilligan is responsible for manuscripts from the Society for Gynecologic Investigation. *Manuscripts from the Society for Gynecologic Investigation should be submitted to Dr. Roger A. Lobo, Women's Hospital, 1240 North Mission Road, Room 1M2, Los Angeles, CA 90033.*

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Abstract page and key words/phrases. On manuscript page 3, type the abstract, double-spaced, with the required margins and headed by the title of the article and name(s) of author(s). Abstracts for regular articles, Current Investigation, Clinical Opinion, and Current Development may not exceed 150 words. Abstracts for case reports and brief communications may not exceed 50 words. Below the abstract list 3 to 5 *key words* or short phrases for indexing purposes.

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

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In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

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—Tables are cited in sequence in the text.

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—Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

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THE GOOD THINGS SHE DOES FOR HERSELF, SHE STAYS WITH…

She stays with TRIPHASIL®
for the same reasons
she started with it:

- Minimal breakthrough bleeding from Day 1
- Low estrogen dose
- Low incidence of common OC side effects*

Triphasil®

Levonorgestrel and ethinyl estradiol tablets—
Triphasic regimen 21- and 28-day regimens

THE OC TO START WITH BECAUSE SHE'LL STAY WITH IT

Worldwide Leadership in Female Healthcare
WYETH-AYERST LABORATORIES
Philadelphia, PA 19101

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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.

See brief summary on adjacent page.

Triphasil®

Levonorgestrel and ethinyl estradiol tablets—
Triphasic regimen 21- and 28-day regimens

THE OC TO START WITH BECAUSE SHE'LL STAY WITH IT

IN BRIEF:

TRIPHASIL® — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen) — Triphasic regimen.

Indications and Usage — TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

> **Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.**
>
> The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days. For full details on dosage and administration see prescribing information in package insert.

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EDITORIAL

Structured abstracts

After one year of thoughtful discussion the Editors of the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY have decided to introduce structured abstracts. We believe that they will be of benefit to both readers and authors. The reader will have at a glance many of the salient facts about a given article. We would be very disappointed if the structured abstract induced the reader to read only the abstract and feel that he/she had thereby received all the information that the author(s) wished to impart; this is not the case. Background, fact, and discussion that cannot possibly be included in the abstract are contained in the full article. Reading only the essence of an article is like smelling a flower and not taking the time to enjoy the beauty of its color and form; you have deprived yourself significantly.

The structured abstract, limited to 150 words, will have four essential parts: **OBJECTIVE(S), STUDY DESIGN, RESULTS,** and **CONCLUSIONS.** These four parts are to be typed as separate headings. The **objective(s)** section should state in a concise manner why the study was done. In many articles it will be the hypothesis being tested, while in others it should point out why the case or technique is being reported—how it might help the reader diagnose a case or manage a patient. The **study design** section should include the locale of the study, the number and type of human subjects, nonhuman species, or material being studied, the principal test(s) performed, and the types of statistical analyses if appropriate. The **results** are those principal results that confirm or refute your hypothesis. When statistical analyses are mandatory for the report, the statistical validity of the results must be included. The **conclusions** should state in a concise way the effect of the study on patient management, our understanding of basic processes within the organism, or the way in which we teach students or patients; in short, the value of the article to the reader.

It was stated earlier in this editorial that the structured abstract may be of value to the author(s). The principal value that we see is in encouraging the author(s) to identify before or early in the study the hypothesis being tested. If that is the case and the study design is rigorously fit to the hypothesis, authors can avoid submitting incomplete manuscripts for possible publication.

The abstracts for all articles, including society articles, will conform to the above structure, with the exception of Clinical Opinions, Current Developments, brief communications, case reports, and other articles that do not contain a Results section. Abstracts for those articles should follow the standard abstract format. The limit of 150 words still applies, except for case reports and brief communications, which are limited to 50 words.

Home monitoring of uterine contractility

Summary of a workshop sponsored by the National Institute of Child Health and Human Development and the Bureau of Maternal and Child Health and Resources Development, Bethesda, Maryland, March 29 and 30, 1989

George G. Rhoads, MD, MPH, Donald C. McNellis, MD, and Samuel S. Kessel, MD, MPH
Bethesda, Maryland

At an interdisciplinary workshop on home monitoring of uterine activity, participants reviewed the basis of this technology and its use in the identification of women at high risk of preterm delivery and in the prevention of preterm birth. Although the guard-ring devices in current use appear capable of detecting uterine activity, they do not clearly distinguish between Braxton Hicks contractions and the contractions of early labor. There was agreement that women destined for preterm delivery have more uterine activity on average than do other women of comparable gestational age but that there is substantial overlap between the two groups. Thus it is uncertain whether this difference can be used effectively for screening purposes. Conferees agreed that there is considerable evidence that twice-daily monitoring of very-high-risk women, in conjunction with daily nursing support and high-quality obstetric care, may prevent preterm births. Available evidence does not clearly distinguish the contribution of tocodynamometry from that of nursing support. A number of areas were identified in which further research is needed. (AM J OBSTET GYNECOL 1991;165:2-6.)

Key words: Preterm delivery, tocodynamometry, uterine monitoring

For some years physicians have been using external tocodynamometers in labor and delivery suites as a convenient, noninvasive method of assessing uterine activity. A number of studies have shown that the tracings obtained with these devices correlate reasonably with direct measures of intrauterine pressure obtained during active labor and provide good estimates of the frequency and duration of contractions, as well as some indication of intensity.

Because patterns of or frequency of low-grade uterine activity may serve as indicators of impending preterm labor, the use of external tocodynamometers to assess risk and to diagnose labor at an early stage has been an attractive concept. Several manufacturers have developed portable devices that are suitable for home use and that allow the transmission of digitized tracings over telephone lines to a remote site for interpretation. Thus women at high risk of preterm labor can be monitored at home, and the resulting information can be made available for use by their physicians. Such home monitoring has been used primarily in conjunction with substantial nursing support that has included instruction in the use of the monitor and daily telephone calls to assess patient symptoms and to ensure smooth transmission of the tracings. Randomized[1-3] and nonrandomized[4] trials in high-risk women have revealed significant reductions in the frequency of preterm births in those patients who received the combined monitoring and nursing services when compared with those who received standard obstetric care. However, these trials have been subject to criticism because of their small size and their failure to separate the effects of the monitor from those of nursing support.

To clarify what is known and what is not known about home monitoring of uterine activity, the National Institute of Child Health and Human Development and the Bureau of Maternal and Child Health and Resources Development convened a workshop on March 29 and 30, 1989, in Bethesda, Maryland. Participants with backgrounds in physiology, bioengineering, mathematics, obstetrics, pharmacology, and epidemiology reviewed the potential limitations and utility of this ap-

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A complete list of additional participants appears at the end of this article.

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proach to the prevention of preterm labor from the perspective of their various disciplines. Six major areas were discussed: animal studies of uterine activity, sensitivity and standardization of noninvasive sensors of uterine activity, uterine contractions during normal human pregnancy, occasional monitoring of the frequency of contractions during pregnancy as an approach to assessing the risk of preterm labor, early diagnosis of preterm labor by daily monitoring, and aspects of tocolytic therapy that are related to a monitoring program.

The current state of the art

It is well known that poorly coordinated, relatively weak uterine contractions occur throughout pregnancy in all mammalian species that have been studied. In the rhesus monkey these epochs of activity, which have been called uterine *contractures*, are easily distinguished by the accompanying electromyographic and intrauterine pressure changes from uterine *contractions* associated with labor.[4a] Contractures are less well synchronized than contractions and often do not involve the whole uterus. Although similar studies have not been done in women, it is reasonable to conjecture that normal Braxton Hicks activity is of the contracture type. The switch from contractures to contractions in gravid monkeys usually occurs around the onset of darkness for a few days before labor begins. Each night the frequency and intensity of contractions increase. In addition, the time period during which contractions occur is longer. Eventually, if all the other features of labor (especially cervical dilatation) occur, delivery will follow. It is, of course, uncertain whether a similar change in the uterine electromyographic pattern occurs in women, and the monitors that have been available for use in humans before labor have not clearly distinguished between these two types of activity.

The tocodynamometers that are available for human use before the rupture of membranes are external devices that are usually held against the abdominal wall with a belt. The excursion of a central piston that is applied to the skin under spring force is measured. As the uterus contracts, the uterine wall becomes more resistant to indentation by the piston, and the resulting piston movement is recorded electronically. Smyth[5] argued some years ago that it is advantageous to mount the piston in the center of a flat plate (the guard-ring arrangement), and the conferees agreed that this type of instrument provided a better record than did other types of external tocodynamometer. Nearly all published information on ambulatory tocodynamometry concerns the guard-ring device.

Validation of the records from external tocodynamometers has been obtained from women in labor by use of intrauterine pressure catheters. In that setting the external instrument has provided excellent data on contraction frequency ($r > 0.9$) and very good data on the duration of contractions. Some investigators have reported a good correlation between the amplitude of the deviations on the external tracing and internal pressure excursions, but where no internal catheter is present, it is not possible to standardize the amplitude of the external tocodynamometer. The amplitude of the excursions seen in a particular situation is sensitive to the location of the instrument, the tension on the belt, and the adiposity of the individual, among other factors. Contractions that occur before the onset of clinical (true) labor are of relatively mild intensity and can be difficult to distinguish from background "noise." Thus the correlations between the external tracings and actual uterine activity could be substantially lower in the outpatient situation than in the labor room. Improvements in external tocodynamometers or other methods of assessing uterine activity should be sought. A beltless tocodynamometer that is held against the abdominal wall with an adhesive recently has been reported to improve the signal-to-noise ratio, especially during maternal exercise.[6] However, its practicality for routine use at home has not been demonstrated. Changes in other cardiovascular parameters that precede the onset of labor should be studied further.

Difficulty in detecting uterine activity with an external monitor before the onset of labor might be reflected in poor reproducibility in the reading of tracings. Very little information about the reproducibility of either the monitor strips or their interpretation is available. In one carefully standardized study that was conducted with "blind" interpretation of the strips, reinterpretation of 600 1-hour strips by the same individual at a later time yielded exact agreement about the number of contractions per hour 79% of the time and showed agreement within one contraction 95% of the time. These results seem good; however, a number of conferees indicated that reproducibility does not always meet these standards.

It is possible that readers may be better at distinguishing between high-risk and low-risk strips than they are at replicating the number of contractions, especially on strips that have relatively low rates of overall activity. On the other hand, agreement between different individuals might be less than the agreement of one reader with his or her own earlier reading. If the readings cannot be shown to be reliable, their utility in classifying women or in making the early diagnosis of preterm labor would be compromised. The potential use of computer interpretation to attain maximal reproducibility at a much reduced cost is attractive and is probably worthy of developmental effort in the near future.

Several investigators have used external tocodynamometers to monitor the frequency of uterine contractions at various times during the second half of pregnancy.[7-14] Although the extent of standardization of the readings is unclear in several of these reports, there is a surprising amount of consistency in their principal results, which have been as follows: The mean frequency of contractions tends to rise with increasing gestation, except for a minor plateau or dip around 31 to 32 weeks; there is an increase in the frequency of contractions 24 to 48 hours before the onset of labor, whether the labor is preterm or term; at a given gestational age the mean contraction rate of women destined to go into preterm labor is higher than the mean rate of those who are not. Although the overlap between the groups is substantial, a few studies have suggested that this difference could be usefully exploited to identify high-risk women. Further exploration is needed in this area. Additional investigation should attempt, among other things, to clarify to what extent contraction frequency is correlated with other risk factors for preterm labor and to what extent it can provide additional information about the risk of preterm labor that is statistically independent of the usual risk measures.

A number of small, randomized trials have been carried out to assess the efficacy of home uterine activity monitoring in actually preventing preterm birth. In most of these trials very-high risk patients such as those with a twin pregnancy, a uterine anomaly, or a history of preterm delivery have been recruited and instructed about the signs and symptoms of early labor and have been trained in the use of the home monitor. These patients have then monitored themselves at home twice a day (morning and evening) and have transmitted the digitized tracings over the telephone in the evening for central interpretation. The results of the central interpretation and any smptoms during the day are discussed by telephone with a nurse and, if necessary, a third hour of monitoring provides clarification. Patients with atypical symptoms or tracings are referred to their physicians and in many instances are evaluated for preterm labor. An encouraging feature of this strategy has been its modest demand on physician time. The trials usually have shown that fewer than one additional unscheduled visit per pregnancy has resulted from participation in the monitoring program.

Several of these randomized trials have compared the monitoring regimen and accompanying nursing support with a routine standard of care.[1-3] In general, these studies have shown no difference in the frequency of diagnosed preterm labor, a marked increase in the proportion of patients with preterm labor in the monitored group in whom the cervix is considered to be favorable for tocolysis, and a substantial reduction in the monitored group in the frequency of preterm birth. These results appear impressive because the studies used a rigorous randomized design and actually achieved a reduction in preterm births, not merely a difference in cervical status at the time of diagnosis of preterm labor. Similar results have been reported in women whose pregnancies were managed at home after a documented episode of preterm labor earlier in the pregnancy.[15] The finding that the cervix was more often favorable for tocolysis in the intervention group was consistent with expectation and suggested that labor was being diagnosed earlier, which allowed more effective tocolysis.

The main limitations of these trials are as follows: They are of limited size; they have been conducted in a very special group of patients who constitute a modest proportion of the preterm birth problem; investigators failed to show results for the modest number of women randomized to tocodynamometry who failed to use the method; they leave unanswered the question of whether the treatment effect is due to the monitoring per se or whether it might be due to the nursing support or the extra periods of limited activity that may accompany the monitoring process. A less important, unavoidable design problem is that the treatment assignments in these trials cannot be masked. In spite of these limitations the finding that the combined nursing and monitoring program has led to a substantial reduction in preterm births in these very-high-risk women remains a striking, perhaps unprecedented result.

Several investigators have tried to assess the effects of the monitor, as opposed to the effects of nursing support and other aspects of the monitoring protocol, by conducting trials that compared the monitored group with control groups who received equivalent nursing support but no monitoring.[16-20] Although the details of most of these trials are still unpublished, they have not shown a convincing positive effect of monitoring. This may suggest that nursing support alone may be sufficient to produce a beneficial effect in high-risk women. Only one of the trials incorporated a direct comparison between routine care and extra nursing support (with sham monitoring). Whereas the differences found suggested that daily provider-initiated nursing support was effective, the numbers were too small to lead to firm conclusions.[16]

Recommendations for further research

The conferees agreed that the animal and human studies suggesting a change in the frequency and quality of uterine contractile activity that occurs a day or two before labor, the evidence that women destined to go into preterm labor have more contraction activity than women who labor at term, and the prima facie case for efficacy of the monitor in combination with vigorous nursing support all suggest the importance of continuing to investigate the role of monitoring in the prevention of preterm birth. However, the evidence that monitoring per se provided an important contribution either to assessing the risk of preterm labor or to preventing preterm birth was thought by most participants to be less than fully convincing. It appears that additional work is needed both to optimize the ability to conduct home uterine activity monitoring and to demonstrate unequivocally that, in its currrent form, monitoring adds to our ability to prevent preterm birth.

The following studies were suggested:

1. Animal research, and particularly work in nonhuman primates, could be used to explore the conditions and physiologic mechanisms that precipitate or inhibit uterine contractions. This work might provide clues about the best time and conditions for monitoring in high-risk women.

2. Studies in animals (or possibly in humans) needs to be done to produce a better sensor for uterine activity. A detection system that could distinguish between Braxton Hicks contractions and the contractions of early labor would be especially desirable.

3. Studies should be undertaken to assess the sensitivity of external monitors to uterine contractions in women before term who are not in active labor. These evaluations could be done in women with maternal indications for induction and could involve simultaneous internal and external monitoring before active labor is established. Alternatively, the studies might be possible in women who are admitted with ruptured membranes but who are not in labor and in whom delivery is planned within a specified time.

4. Additional work is needed to establish the reproducibility of the reading of tracings obtained from external monitors before labor. An attempt to develop reliable and valid computer interpretations of the tracings should be a high priority. The effect of patient activity and the extent of adiposity on the quality of the tracings need further evaluation. The quality of tracings from different devices also should be formally compared. When patients are monitored repeatedly over a number of days, mathematic approaches to the interpretation of the accumulating data deserve further exploration. Some of this work could be done with tracings that have already been collected.

5. The utility of monitoring contraction frequency in clinic and other outpatient settings as an approach to assessing patient risk status needs further evaluation. These evaluations should be done in contexts in which the quality of the interpretation of the tracings is well documented and the information on other risk factors is available, so that the magnitude of the additional contribution of the monitor information to the prediction of preterm labor or birth can be estimated.

6. A large, randomized trial is needed to verify the effectiveness of the combination of monitoring and nursing support in reducing the incidence of preterm birth in high-risk women. This trial should have several study groups, so that the effect of nursing support without monitoring can be evaluated separately. Some conferees felt that it would also be worthwhile to evaluate the effect of monitoring without intensive nursing support. The question of whether such a trial should be undertaken now or should wait for the completion of some of the projects outlined above was not resolved and would obviously depend in part on the availability of resources.

7. A separate clinical trial might be considered to test the efficacy of monitoring in women who have had an episode of preterm labor and who are at home on a regimen of tocolytic therapy. Additional work on the efficacy of oral tocolytics as compared with subcutaneous infusion of tocolytics might be needed to plan an optimal design for such a trial.

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Screening in ovarian cancer

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Ovarian cancer will affect 20,000 American women this year and some 13,500 will die of the disease. Most patients will have stage III or IV disease at the time of diagnosis. Because ovarian cancer may be initially without symptoms, attempts have been made to develop screening tests that would lead to an early diagnosis. Two tests, serum CA 125 and pelvic ultrasonography, have been suggested by some to be accurate enough to be included in yearly screening for ovarian cancer. Although further testing of these techniques is encouraged, data to date do not justify yearly screening with CA 125 or pelvic ultrasonography in all women. (AM J OBSTET GYNECOL 1991;165:7-10.)

Key words: Ovarian cancer, screening

With the untimely death of Gilda Radner because of ovarian carcinoma, this malignancy has become the cancer of the year. The media have given it considerable publicity with at least two news commentary programs having major segments on this subject. Both featured Gilda Radner's plight. Unfortunately, without her death the public would not have received important information about this cancer. However, awareness alone does not lead to optimal management.

The publicity has emphasized the familial associations and as a result suggested new screening guidelines for this cancer. It has been known for some time that relatives of patients with ovarian cancer have an increased incidence of this cancer although a specific genetic link has not been identified. This appears to be particularly true if two or more family members (first-order relatives) have had ovarian cancer. The familial ovarian cancer registry has accessed 1020 ovarian cancer patients. Obviously this is a highly select group of patients. Recent data from this registry has not been reported in the peer-reviewed literature and only briefly in a 1990 newsletter. Unfortunately, the denominator is unknown. A recent study by Koch et al.[1] evaluated family history of ovarian cancer patients with a case-control study. They identified 197 patients with ovarian cancer diagnosed between Jan. 1, 1984, and March 31, 1986, and 210 control subjects from Alberta, Canada. Because of a population-based cancer registry they were able to include >95% of cancer cases in Alberta and verify the relatives' cancer history. They noted that the family cancer history given by the patient often was inaccurate as to the cancer site. There was a significantly higher incidence of ovarian cancer in the family history of these patients compared with the control group but it is still not very common. This finding may be falsely elevated inasmuch as the control subjects had fewer siblings than did the ovarian cancer patients. Of the 197 cases only 9 (4.6%) had a positive family history of ovarian cancer; only 1 patient with ovarian cancer (0.5%) had more than one relative with ovarian cancer and that patient came from a family of nine sisters. It has been estimated that only 1% to 3% of patients with ovarian cancer fall into this category. Although important for those women who are at risk, family history apparently affects only a small minority of patients.

It has been suggested that all women should have a periodic pelvic examination, pelvic ultrasonographic examination, and CA 125 screening to make sure they do not harbor an occult ovarian cancer. Most would agree that a bimanual rectovaginal examination should be part of the annual physical, although it has been stated that one has to do 10,000 routine examinations to pick up the one early ovarian cancer. That is one of the problems with this cancer—the incidence is relatively low with no known preinvasive lesion. There are approximately 20,000 new ovarian cancers each year compared with approximately 150,000 breast cancers. For a screening test to be effective there needs to be an appreciable prevalence, an identifiable preinvasive lesion, and an effective screening test with high sensitivity and specificity. With that caveat in mind, we examined ultrasonography and CA 125 as screening tools.

CA 125

The use of CA 125 as a screening technique is being evaluated in Stockholm. In 1990 a preliminary report was published.[2] A total of 1082 women aged 40 years or older had serum CA 125 levels determined. Thirty-six (3.3%) had levels >35 U/ml and 11 (1.0%) had levels >65 U/ml. Although several of these women continued

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to have elevated CA 125 levels, only two had a doubling of these levels and in only one was this increase sustained. Twenty-one months after the initial elevated CA 125 levels (35.3 U/ml) were recorded and 15 months after the first doubling (69.7 U/ml) a stage III mixed mesodermal ovarian cancer was diagnosed.[3] With the use of the "doubling rule" the specificity of the assay for one ovarian sarcoma was 99.9%. The specificity was 100% for a "sustained increase rule" (positive for the doubling rule, the levels continue to rise progressively for an additional period of time). The predictive positive and negative values would have been lower with the initial values. The yearly incidence of ovarian cancer in Sweden in the age group tested is about 30/100,000 population and perhaps an additional 30 cases prevalent but not clinically manifested. If prevalence of the disease is 30 to 60 cases per 100,000 then even with a specificity of 99.5% only 6% to 11% of all women with elevated CA 125 levels would actually be expected to have cancer even if the sensitivity was 100%. A subsequent abstract[3] updated this experience. A total of 5550 women were measured annually. Among 175 women with elevated CA 125 levels, six ovarian cancers were detected clinically or ultrasonographically. Four of the six patients had stage IIB or III ovarian cancers. The authors stated "A prospective controlled trial will be required to determine whether such screening reduces mortality."

A retrospective study by Zurawski et al.[4] from Norway evaluated CA 125 from the JANUS serum bank, which is a repository of specimens collected since 1973 from more than 100,000 persons. The authors were able to identify 105 patients who had banked serum and in whom an ovarian malignancy subsequently developed. They compared these results with 320 matched control subjects. The authors identified 12 patients who had blood drawn ≤18 months before the diagnosis. One of the patients had a tumor of low malignancy potential. Only four of these 12 had CA 125 levels >35 U/ml. Three of these had localized disease. One patient had a diagnosis made 1 month after the blood was drawn and she had disseminated disease. Whether the three patients with localized disease could have had the diagnosis established at an earlier time because of the screening for CA 125 is obviously unknown. No data are given as to the subsequent course of these three patients. In another study, Jacobs et al.[5] tested 1010 postmenopausal volunteers with CA 125. One case of ovarian cancer was detected by screening; the CA 125 level was 32 U/ml. No other patient has presented clinically after about 2 years of follow-up. With the use of a 30 U/ml cutoff, the detection rate was 100%, the false-positive rate was 3%, and the odds of being affected given a positive result (the ratio of the number of true-positives to the number of false positives) was 1:30.

The use of CA 125 has been evaluated in an attempt to distinguish benign from malignant masses in patients with known pelvic masses. Malkasian[6] evaluated CA 125 levels in 158 patients with palpable pelvic masses. Sixty-eight were found to have cancer with the other 90 benign. In the latter group 72 (80%) had CA 125 levels <35 U/ml. In the 68 patients with cancer, 21% had CA 125 levels <35 U/ml and 17 (25%) had levels <65 U/ml. If the CA 125 level of 65 U/ml was the cutoff value, the sensitivity was 60% and specificity was 89% in the premenopausal group and 78% and 97%, respectively, in the postmenopausal group. Patsner and Mann[7] evaluated 250 consecutive patients with pelvic masses. Of those with benign disease, 16/74 (21%) who were premenopausal had CA 125 levels >35 U/ml. In the postmenopausal patients with benign disease 9/48 (18%) had elevated CA 125 levels. Of those with malignant gynecologic cancers, 63% of the premenopausal patients and 77% of the postmenopausal patients had elevated CA 125 levels. Sensitivity for CA 125 was 0.65 and 0.77 for premenopausal and postmenopausal patients, respectively; specificity was 0.78 and 0.81, respectively. Positive predicted value was 0.67 in the premenopausal patients and 0.87 in the postmenopausal patients, whereas the negative predictive value was 0.77 and 0.68, respectively.

Finkler et al.[8] correlated CA 125 levels in patients with adnexal masses singularly and in combination with clinical impression as well as with initial and review ultrasonography. They evaluated 102 consecutive patients with adnexal masses. A clinical impression was determined preoperatively by the examiner as to whether a mass was thought to be benign or malignant. A pelvic ultrasonographic examination was performed and impression of benign or malignant was given by the initial reviewer. Ultrasonographic scans were then reviewed by the "expert" who had very strict criteria for the designation of malignancy. Eighteen of 70 (24%) of the premenopausal patients and 19 of 32 (59%) of the postmenopausal patients had a malignancy. In the premenopausal patients the predictive positive and negative value for CA 125 was 36% and 82%, respectively, which was comparable to the clinical evaluation and the initial ultrasonographic review. In the postmenopausal patients the predictive positive and negative values for the CA 125 was 94% and 80%, respectively. Predictive positive value of the clinical and initial ultrasonographic review were similar; however, the CA 125 did have a better predictive negative value than did the clinical or initial ultrasonographic review.

An interesting study of 4000 volunteers has been reported from Kings College in London.[9] The patients were older than 44 years of age and were postmenopausal. They underwent a vaginal examination and a CA 125 evaluation. Those persons with an elevated CA 125 level (>30 U/ml) or those who had abnormal examination results were recalled for abdominal real-time ultrasonography. If the ultrasonographic examination revealed an ovarian enlargement, patients were further investigated surgically. It was noted that in 3839 patients the CA 125 screening and pelvic examination results were normal and an ultrasonographic examination was not performed. In the second group all 69 patients had elevated CA 125 levels; however, the pelvic examination results were normal. Four patients had abnormal ultrasonographic results and at the time of surgery no cancers were noted. The third group of patients comprised persons who had abnormal pelvic examination results and normal CA 125 levels. In this group of 91 women, 19 had abnormal ultrasonographic results and in the 19 who underwent surgery no cancers were noted. In the last group, there was only one patient who had an elevated CA 125 level (32 U/ml) and abnormal pelvic examination and ultrasonographic results. She was found to have stage IA clear cell carcinoma of the ovary. None of the other patients in the study has had documented ovarian cancer after a minimum follow-up of 12 months. In this study the CA 125 level and the pelvic examinations were both highly specific (97% and 97.3%, respectively). If a patient had an elevated CA 125 level and either abnormal pelvic or ultrasonographic examination results, specificity was 100%. Finkler[6] noted in his study that in the postmenopausal patient with an abnormal CA 125 level that if either the pelvic or ultrasonographic examination was positive, the positive predictive value was 100%.

Ultrasonography

A study from England[10] has been reported in which the value of ultrasonography in the screening procedure for early ovarian carcinoma was evaluated. Almost 5500 women were self-referred and underwent a first screening. Ninety percent of these had a second screening and three fourths had a third screen. Eventually 5479 women were monitored over 8 years with 14,594 screenings (15,977 scans) and 29,140 ovaries examined. The median interval between the first and second screening was 614 days and 564 days between the second and third screening. These women were aged 45 years or more and could be either premenopausal or postmenopausal. The authors of this study are considered to be leading authorities in ultrasonography for obstetrics and gynecology worldwide. They use three criteria for designation of a positive scan: ovarian morphology (hyperecogenicity or hypoecogenicity), outline (irregular), or volume (>20 ml). The ovaries had to be identified to give a negative or positive scan. Women with normal scan results were then asked to return 1 year later. Those with an abnormal result were rescanned after 3 to 8 weeks to exclude any transient change in morphology or size. A total of 14,594 patients were screened from one to three times. A total of 654 scans were positive initially but became negative on rescanning; 338 scans were positive both on the initial and the rescan. A total of 326 women (from 338 scans) underwent exploratory laparotomy. Five patients had primary ovarian cancer; three were classified as borderline. All the primary cancers were stage IA or IB. All these patients are alive at least 2 years after surgery. Three of the primary cancers were detected at second screening at 16, 18, and 22 months after the first screening. In addition, there were four patients with metastatic ovarian cancers. The authors stated that they were unable to identify any morphologic characteristic or condition that could be used to differentiate between benign and early malignant tumors. The overall specificity of the screening procedure was 97.7% and predictive value for positive result was 1.5%. Therefore the odds of having a primary ovarian cancer in a patient with a positive result was only 1:67, indicating that 66 of the 67 patients who underwent exploratory laparotomy had no evidence of a borderline or invasive primary cancer. There was an additional 186 patients who declined surgical procedures or became nonattenders. Because cancer had not become apparent at least clinically in this latter group of patients, the odds of positive finding would decrease even more than the 1:67 the authors actually noted. These authors are proceeding to a very large screening protocol to determine whether ultrasonography is a useful screening tool and to determine cost effectiveness. Obviously the benefit of using this modality as a screen for ovarian cancer has not been proved and further evaluation may be justified.

Prevention of ovarian cancer

It should be remembered that ovarian cancer can be markedly reduced with the use of oral contraceptives. Fourteen studies in the literature all show a decrease risk. After 4 years of use, there is a 50% reduction and with 7 or more years there is a 60% to 80% reduction in ovarian cancer. Obviously such prevention would appear to be more effective in lowering the incidence and death rates of this cancer than currently available diagnostic tests of our current therapy.

Comment

In the small group (<5%) of high-risk patients, a CA 125 and pelvic ultrasonographic screening may be periodically justified. There is no data to support this action; however, it may be acceptable if for no other reason that to reassure these patients.

It is estimated that there are more than 43,000,000 women in the United States aged 45 years or older. A common cost of nonobstetric diagnostic pelvic ultrasonographic examination is $275.00. A CA 125 screen-

Predictive value of early embryonic cardiac activity for pregnancy outcome

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The biologic variation of the embryonic heart rate was investigated by transvaginal ultrasonography during the first trimester of normal ($n = 141$) and abnormal ($n = 29$) pregnancies, and the predictive value of a repeatedly diagnosed slow heart rate for pregnancy outcome was determined. The mean heart rate increased from 82 ± 10.3 beats/min at 5 weeks' gestation to 156 ± 9.6 beats/min at 9 weeks. A single observation of an abnormally slow heart rate did not necessarily predict subsequent embryonic death. However, a continued decline in embryonic heart activity, observed within a few days' interval, was always associated with a first-trimester abortion. (AM J OBSTET GYNECOL 1991;165:11-4.)

Key words: Fetal heart rate, first-trimester abortion, transvaginal ultrasonography

The earliest proof of a viable pregnancy is obtained when cardiac activity of the embryo can be observed. High-frequency vaginal transducers have improved embryonic imaging in early pregnancy and have facilitated the very early detection of cardiac activity. Several investigators[1-3] have reported a gradual increase in embryonic heart rate as detected by ultrasonography starting in the fifth week of pregnancy. Hence, a heartbeat frequency hovering around 100 beats/min may be a physiologic phenomenon in early pregnancy.[2,3] There is evidence, however, that an abnormally slow heartbeat is associated with impending embryonic death,[1] but the predictive value of embryonic heartbeat frequency for the evolution of early pregnancy is still unknown. Therefore it was the aim of this study to define the evolution and the biologic variation of early embryonic heart rate between the fifth and twelfth weeks of pregnancy and to correlate abnormally slow heart rates with pregnancy outcome.

Material and methods

This study was performed over a 6-month period on 170 pregnancies (fifth through twelfth weeks, 552 observations) examined in our ultrasonography department. Patients were referred from either our outpatient or infertility clinic. Of these 170 patients, 146 conceived spontaneously and 24 became pregnant after gamete intrafallopian transfer ($n = 5$), in vitro fertilization and embryo transfer ($n = 16$), or zygote intrafallopian transfer ($n = 3$). Patients were included only when follow-up beyond the first trimester of pregnancy was available and when gestational age was accurately determined by basal body temperature chart, luteinizing hormone surge, or date of ovum pickup. The heart rate was measured by counting heartbeats several times for periods of 15 seconds, averaging, and multiplying by four. All scans were done by the same observer using a 5.0 or 7.5 MHz ATL (Ultramark) vaginal transducer.

Results

Of the 170 patients recruited in this study, 141 (14 in vitro fertilization, 5 gamete intrafallopian transfer, 2 zygote intrafallopian transfer) were pregnant, and 29 experienced a miscarriage before week 12. One of those miscarriages was ultrasonographically and histologically diagnosed as hydatidiform mole. In the uncomplicated pregnancies fetal cardiac activity could be detected as early as 5 weeks 3 days (20/24 observations). Between 5 weeks 3 days and 6 weeks cardiac activity was registered in 36 of 42 embryos. Cardiac activity was always detected in the uncomplicated pregnancies from 6 weeks 1 day on (345 observations). Although the em-

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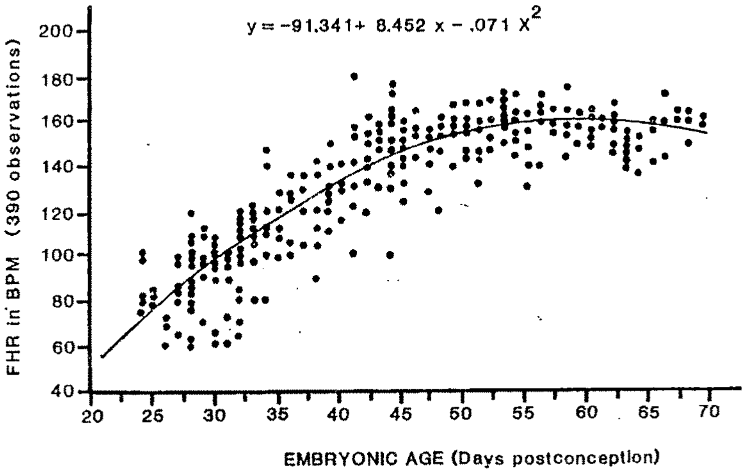

Fig. 1. Embryonic heart rates in 141 normal first-trimester pregnancies (390 observations).

Table I. Mean embryonic heart rates in first trimester of pregnancy (141 normal pregnancies)

Gestational age (wk)	*No. of examinations*	*FHR (beats/min, mean ± SD)*
5	47	82.41 ± 10.34
6	77	95.83 ± 18.85
7	66	125.35 ± 18.30
8	65	146.68 ± 15.13
9	56	156.23 ± 9.63
10	40	157.12 ± 8.13
11	25	153.68 ± 8.79
12	25	152.56 ± 9.06

Spearman rank correlation: $R = 0.80$, $p < 0.001$.

bryonic heart rate varied considerably between the sixth and twelfth weeks of gestation, ranging from 60 to 170 beats/min, the mean fetal heart rate (FHR) increased significantly from 82 beats/min (fifth week) to 156 beats/min (ninth week) (Table I, Fig. 1).

Not included in these calculations is the extremely low cardiac activity we observed on a single occasion at 7 weeks' gestation in an otherwise uneventful pregnancy. A FHR of 48 beats/min persisted for >5 minutes. We recorded the entire observation period on videotape. The maternal radial pulse at that moment was 80 beats/min. This disquieting image disappeared spontaneously and could not be reproduced on reinsertion of the vaginal probe a few minutes later.

An embryo could be visualized in 26 of 29 cases of subsequent first-trimester abortion. In 10 of these cases heart activity was never detected, whereas in the remaining 16 heart activity was recorded on several consecutive occasions with an interval of 3 to 4 days. In nine of these 16 cases the maximum FHR did not exceed 100 beats/min, and in 10 we recorded a gradually declining heart activity at repeated scans with an interval of 2 to 3 days, until heart activity could no longer be observed (Fig. 2). The heart rates of what we called "dying embryos" ranged from 52 to 100 beats/min. A heartbeat was always detected after 6 weeks 1 day in the normal pregnancies; therefore an absent ($n = 10$) or delayed ($n = 5$) appearance of the FHR from that moment on invariably predicted abortion (sensitivity, 100%; specificity, 100%). The specificity, sensitivity, and predictive value for different cutoff levels of FHR at week 7 are given in Table II.

Comment

The cardiovascular system is the first system to function in the embryo.[4] During embryonic development the FHR develops from splanchnopleuric mesoderm that forms a horseshoe-shaped area in the anterior part of the embryonic disk, part of the secondary yolk sac wall. In this region two midline endothelial heart tubes form (18 to 19 days after conception) and fuse to form a single heart tube (22 days after conception; 5 weeks 1 day of pregnancy). At that very moment the heart begins to contract and has a peristaltic wave motion.[4] Before 22 days after conception it is impossible to detect cardiac activity by ultrasonography. The atrium and ventricle move blood in an ebb-and-flow fashion. By the sixth week of pregnancy the flow becomes unidirectional and cardiac contractions have become coordinated. Between 5 and 6 weeks' gestation, the body folds constrict the yolk sac, and the part incorporated into the embryo forms the epithelium of the gut tube.[4] The remainder of the yolk sac (visible ultrasonographically) is adjacent to the embryo and connected to it by the yolk stalk immediately caudal to the fetal heart bulge, just before and at 6 weeks' gestation. Because of this relationship, we used the yolk sac as an anatomic landmark when searching for early embryonic heart activity. At 6 weeks the embryo is approximately 4 mm.

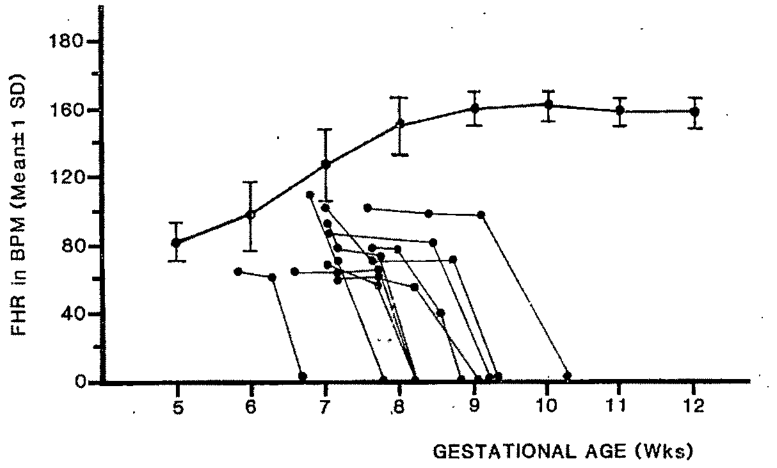

Fig. 2. Mean FHR (± 1 SD) as a function of gestational age in 141 normal pregnancies. Upward trend of normal heart rates between 5 and 9 weeks is demonstrated. On this graph individual *solid lines* represent consecutive FHR registration of dying embryos, which are characterized by a "vanishing heart activity" pattern.

Table II. Operating characteristics at different FHR cutoffs at week 7

FHR (beats/min)	*Sensitivity (%)*	*Specificity (%)*	*Positive predictive value (%)*	*Negative predictive value (%)*
120	96	62	50	97
100	88	88	75	95
80	71	98	94	89

Barry[5,6] studied the developing chick heart and demonstrated that the initial heart rate was very slow under physiologic conditions. The rate increased as the atrium differentiated and assumed a pacemaker function. Several earlier ultrasonographic studies regarding embryonic heart rates demonstrated an upward trend in heart rate early in the first trimester.[1-3,7,8] The pioneer study of Robinson and Shaw-Dunn[8] of the embryonic heart rate after the sixth week of pregnancy revealed that the rate increased from 123 beats/min at 45 days to a peak of 177 beats/min at 15 weeks. Shenker et al.[3] described heart rates of 90 beats/min at 6 weeks and 124 at 7 weeks. The biologic significance of abnormally slow embryonic heart rate has not yet been extensively investigated. Fine et al.[9] described a gradual increase in normal heart rates between 6 and 9 weeks but also reported one case of bradycardia resulting in spontaneous abortion. Hertzberg et al.,[2] however, emphasize that slower heart rates are normal in early pregnancy and warn against their misinterpretation as an abnormal finding or their confounding with maternal pulsations. In a series of 56 viable pregnancies, Laboda et al.[1] found normal first-trimester FHRs to rise from an average of 100 beats/min at 5 to 6 weeks to 140 beats/min at 8 to 9 weeks. None of the ongoing pregnancies had an FHR <90 beats/min. According to these investigators, an FHR <85 beats/min invariably was associated with a poor prognosis. The results of our study confirm the gradual increase of fetal heart rate between 5 and 9 weeks' gestation. Fetal cardiac activity on vaginal ultrasonography is a reassuring sign, but there is still a risk of early pregnancy loss. Although abnormally slow cardiac activity may be associated indeed with impending embryonic death, the predictive value of a single observation at different cutoff levels (e.g., 100 beats/min) is too low to be of any clinical value (Table II). It can provide a warning signal and an indication to perform a repeat scan at a later date. If the FHR declines further on subsequent scans, eventual embryonic death is highly probable. In our series an overall sensitivity of 83.4% and specificity of 97.9%

Table III. Operating characteristics for decreasing FHR pattern and cutoff level of 100 beats/min as predictor of spontaneous abortion between weeks 5 and 10 of pregnancy

True positive: 10	False positive: 3
True negative: 139	False negative: 2
Specificity: 97.9%	Sensitivity: 83.4%
Positive predictive value: 76.9%	
Negative predictive value: 98.6%	

was noted when a cutoff value of 100 beats/min was combined with a declining FHR (Table III).

If we focus on the same criteria in the seventh week of pregnancy, sensitivity and positive predictive value increase to 90.8% and 83.4%, respectively. On the basis of these findings, the seventh week of pregnancy seems to be the most appropriate time to predict the outcome of early pregnancy with a combination of declining FHR and FHR cutoff at 100 beats/min.

It can only be speculated whether the association between impending embryonic death and slow heart rate is a general manifestation of poor embryonic development or, at least in some cases, results from a primary cardiac defect. Shenker et al.[3] ascribe the slower heart rates in early pregnancy to the immaturity of the sinoatrial node or to slower intrinsic atrial pacemaker activity. On the other hand, embryonic bradycardia may reflect idioventricular rates in an abnormally developing heart, or it may be due to an arrest in the development of the conduction system.

The single case of extreme but transient bradycardia (48 beats/min) in an eventually normal pregnancy, however, illustrates that physiologic mechanisms also can play a role. In this patient the uterus was extremely anteverted and the bladder was empty. When the ultrasonographic transducer is pressed too firmly on the vaginal and thus on the uterine wall, an increase in intrauterine pressure results; if so, the increased intrauterine pressure is transmitted throughout the intrauterine cavity and on all parts of the embryo, including the umbilical vessels. As a result, a primitive efferent vasodepressor response could occur through the vagal center. This phenomenon also was described by Mendoza et al.,[10] who induced fetal bradycardia in midpregnancy by applying pressure to the maternal abdomen during ultrasonographic examination.

In conclusion, use of the transvaginal probe has made it possible to detect embryonic heart activity as early as 5 weeks 3 days of pregnancy. A gradual heart rate increase from 82 beats/min at 5 weeks to 157 beats/min at 10 weeks is a physiologic phenomenon related to the development of the embryonic heart and conduction system. Hence the single observation of a slow FHR has no clinically significant predictive value for the outcome of early pregnancy. On the contrary, a repeatedly detected abnormally slow heart rate signals delayed and poor embryonic development or an abnormally developing heart and predicts subsequent miscarriage. In rare cases, however, this pattern may be the manifestation of a defective heart conduction system.

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Double jeopardy: Twin infant mortality in the United States, 1983 and 1984

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The United States Linked Birth/Infant Death Data Sets: 1983 and 1984 Birth Cohorts from the National Center for Health Statistics were used to identify maternal and infant characteristics related to twin infant mortality; 41,554 white and 10,062 black live-born matched twin pairs were evaluated. Twin birth weight distribution was skewed with 48% of white and 63% of black twins born weighing <2500 gm. Overall infant mortality rates were 47.1 and 79.3 deaths per 1000 live births for white and black twins, respectively (five times the rates for singletons). Three fourths of deaths were among twins weighing <1500 gm. White like-gender twins had about twice the risk of both twins dying compared with unlike-gender twins. Likewise, white twin pairs with >25% birth weight disparity had a 40% to 80% increased risk of both twins dying, compared with twins whose weights were within 10% of each other. Twins born to high-risk women (on the basis of demographic factors) were twice as likely to die as twins born to low-risk women. Thus strategies to decrease twin infant mortality must address both maternal and infant risk factors. (AM J OBSTET GYNECOL 1991;165:15-22.)

Key words: Twins, infant mortality, risk factors

From conception, human twinning is a high-risk event. On the basis of ultrasonographic studies, one fetus vanishes by the end of the first trimester in approximately 20% of twin pregnancies.[1] Problems later in pregnancy include high rates of toxemia and increased risk of fetal death, premature delivery, and intrauterine growth retardation, when compared with singleton pregnancies.[2] Thus, although twins represent only 2% of live births, they account for approximately 10% of perinatal deaths.[3] In addition, infant death rates among twins are estimated to be four to 10 times those of singleton births.[4-6]

In spite of the documented risk of increased infant mortality among twins, data on factors associated with this increased mortality are inconsistent. Previous research has often been limited by small sample size and lack of generalizability of the study populations. For example, some researchers have shown higher neonatal mortality for second-born twins,[7, 8] but others[2, 9] have not. Findings on mortality rates of like-gender versus unlike-gender twin pairs also have varied, although the majority of studies[4, 9, 10] indicate that monozygotic twins (particularly monoamniotic twins)[11] have higher mortality rates than dizygotic twins.

Likewise, although congenital anomalies are known to be a leading cause of infant deaths among twins, studies disagree regarding rates of congenital anomalies among twins compared with singletons[1, 4] or differences in congenital anomaly rates among like- versus unlike-gender twin pairs.[1, 4, 7]

In addition to these infant factors, increased infant mortality among twins probably is also related to the limited adaptability of the human uterus to accommodate multiple fetuses.[7] Furthermore, twinning is associated with other recognized maternal risk factors for infant mortality, such as advanced maternal age and parity and black ethnicity.[2]

In view of the limitations of previous research on twin infant mortality, this study of national vital statistics was undertaken to evaluate factors related to infant mortality among black and white twins. The Linked Birth/Infant Death Data Sets: 1983 and 1984 Birth Cohorts from the National Center For Health Statistics (NCHS), representing the cohort of all live births in the United States in 1983 and 1984 and any subsequent infant deaths among the cohort, were used for the analyses. Black and white twins were studied separately. Infant mortality was chosen as the outcome for the analyses, since with neonatal intensive care the timing of deaths related to perinatal problems often extends beyond the neonatal period.

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Methods

Data were obtained from the Linked Birth/Infant Death Data Sets: 1983 and 1984 Birth Cohorts. Infant death records from 1983 or 1984 NCHS mortality files were linked with 1983 NCHS birth records, and infant death records for 1984 and 1985 were linked with 1984 United States birth records. The data files include birth and death records of United States residents that occurred in the United States but exclude births and deaths of United States residents occurring outside the United States.

The focus of several of the analyses presented here was on twin pair characteristics, rather than on individual twins. Thus every effort was made to match cotwins from the same pregnancy. Live births in twin deliveries were matched with respect to county and state of birth, maternal race, age, county and state of residence, prior live births, month prenatal care began, education, and martial status as well as father's race, age, and education, to identify twin pairs. Of the 135,051 twins on the 1983 and 1984 birth files, 105,576 (78%) were successfully paired. Of the remaining unmatched 29,475 twins, 1939 were omitted because more than two records had identical matching variables and 27,536 had no matching birth. Of the latter, 11,458 were born in one of four states (Arizona, California, Delaware, Georgia) or Washington, D.C., which included only a 50% sample of births (for which no match of twin pairs would be expected). The primary reasons for failure to find a match for the remaining 16,078 twins included inconsistent birth order data and the possibility of fetal death of the other member of the twin pair. The infant mortality rates that were based on the matched pair data file were 1.1% lower for white and 1.2% lower for black twins than the rates that were based on the unmatched twins. Thus the omission of the unmatched twins does not appear to introduce any substantial bias.

The analyses presented here are based on the 41,544 white and 10,062 black twin pregnancies in which liveborn twins were successfully paired. Some analyses, including birth weight–specific, gender and birth order mortality rates and specific cause of death, were calculated from a data file that included all twin pairs and a selected number of demographic variables. Other analyses (infant mortality rates in relation to maternal risk factors and infant pair characteristics) used a more detailed file, which included all twins pairs in which either or both members died and a 10% sample of pairs in which both members survived.

Infant mortality rates by individual twin infant factors including gender, birth order (twin A vs twin B), and birth weight were calculated for blacks and whites. The relationship of gestation to infant mortality was not presented because of the questionable reliability of last menstrual period–based gestation data.[12]

The relationship of twin pair characteristics to infant twin mortality was assessed for the separate outcomes in which either member or both members of a twin pair died. Twin pair characteristics evaluated were gender pair differences (unlike-gender pairs vs like-gender male pairs or like-gender female pairs) and birth weight differentials between the twin pair members. With respect to gender pair differences, all unlike-gender pairs were obviously dizygotic. However, from the available data, monozygotic twins could not be identified from among the like-gender twin pairs. Birth weight differentials were categorized into five intervals, according to the difference between the twins' weights in grams relative to the average weight of the pair:

The difference (A − B) was >25% of the average weight of the pair (A 25% >B).
The difference (A − B) was 10% to 25% of the average weight of the pair (A 10% to 25% >B).
The difference (A − B) was within 10% of the average weight of the pair (A = B).
The difference (B − A) was 10% to 25% of the average weight of the pair (A 10% to 25% <B).
The difference (B − A) was >25% of the average weight of the pair (A 25% <B).

Summary levels of maternal demographic risk (low, moderate, or high) that had been developed for singletons[13] were analyzed in relation to twin infant mortality. Births with education not stated (primarily births in Texas and Washington where education is not on the birth certificate) were excluded from these analyses. The low-risk group consisted of married primiparous women 20 to 29 years old and low-parity (one prior birth to women <25 years old or two or fewer prior births to women ≥25 years old) multiparous women who were ≥20 years old with ≥13 years of education. The high-risk group consisted of unmarried teenagers, primiparous women ≥30 years old, and high-parity (two or more prior births to women <25 years old or three or more prior births to women ≥25 years old) multiparous women with <12 years of education. The moderate-risk group included all other combinations of maternal characteristics.

Twin infant mortality rates, risk ratios, and confidence limits were computed for black and white twins separately, according to individual twin characteristics (sex and birth order) and twin pair characteristics (like- vs unlike-gender and birth weight differential) for two pair outcomes: both twins died and either twin died. These analyses were done with the BMDP statistical programs.[14]

Cause-specific mortality. Cause-specific infant mortality rates per 100,000 live births were calculated for firstborn and second-born twins with the three-digit and four-digit ICD-9 codes recorded for cause of death, according to the ninth revision of the International Classification of Diseases.[15] From these mortality rates

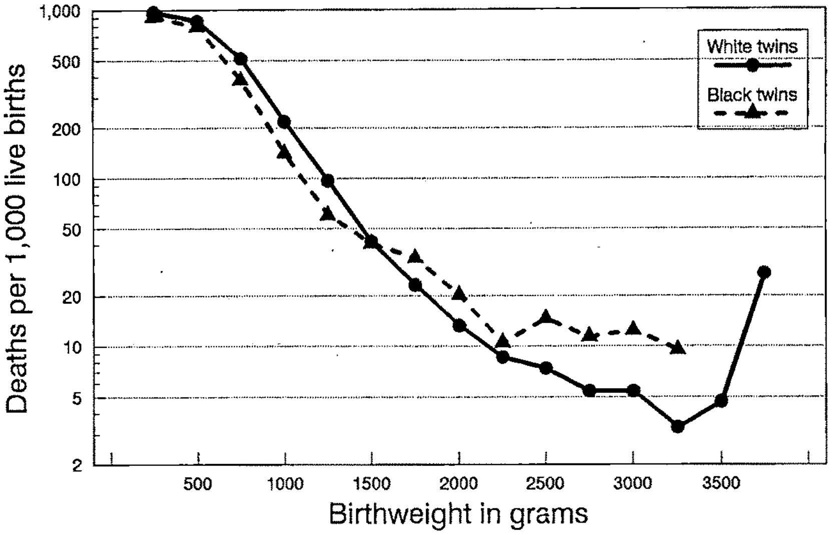

Fig. 1. Birth weight–specific infant mortality rates for black and white twins, from Linked Birth/Infant Death Data Sets: 1983 and 1984 Birth Cohorts for the United States.

cause-specific relative risks for second-born compared with firstborn twins were generated to clarify previous research regarding cause-specific mortality risks of second-born compared with firstborn twins. An obvious caveat regarding these death certificate data is that the information is less accurate than research data obtained directly from autopsy findings; however, these are the only national data regarding specific causes of death that were available to us from the linked files.

Preliminary analyses revealed that the category *Newborns Affected by Maternal Complications of Pregnancy* (ICD-761), which includes *Multiple Pregnancy* as a subcode, was the second most frequently listed cause of death among twins. This was due to a coding rule requiring the choice of ICD-761.5 *(Multiple Pregnancy)* as the underlying cause of twin death instead of ICD-765 *(Short Gestation and Unspecified Low Birthweight)*, even if the physician had listed ICD-765 as the underlying cause. To establish a more meaningful assessment of ICD-761 and ICD-765 among twins, deaths for which ICD-761.5 *(Multiple Pregnancy)* was recorded as the underlying cause of death were recoded to ICD-765 *(Short Gestation and Unspecified Low Birthweight)* if the latter code was listed as one of the multiple causes of death.

Results

The Linked Birth/Infant Death Data Sets from 1983 and 1984 included 41,554 white and 10,062 black twin pairs in which both members were born alive. Approximately two thirds of the twin pairs were like-gender pairs (30,191 white and 6572 black pairs).

Approximately one half of white twin births and two thirds of black twin births were low birth weight (LBW), (<2500 gm), whereas 9% of white and 16% of black twin births were in the very-low-birth-weight (VLBW, <2500 gm) category. This contrasts with national data regarding singleton births in which only about 5% of white and 11% of black singleton births occurred in the LBW category; only 1% of white and 2% of black singleton births were in the VLBW category.[3] Firstborn twins were slightly heavier than second-born twins. Among whites, firstborn twins included 46.5% weighing <2500 gm and 8.5% <1500 gm, compared with 48.7% and 8.7%, respectively, for second-born twins. Among blacks, the differentials were similar: 61.1% <2500 gm and 15.4% <1500 gm for firstborn twins compared with 63.2% and 16.0% for second-born twins.

Infant mortality. There were 3911 white and 1595 black infant twin deaths. Overall infant mortality rates for white and black twins were 47.1 and 79.3 per thousand live births, respectively. These rates are approximately five times those for white and black singletons. However, the black/white mortality ratio is slightly lower for twins (1.68) than for singletons (2.03).[3]

Three fourths of infant twin deaths for both races occurred in the VLBW category. As with singleton births, there was a crossover in the birth weight–specific mortality rates by race. Black twins had a relative survival advantage in the VLBW range and a survival disadvantage above this range (Fig. 1). The lowest mortality rate for white twins was at 3250 gm (3.2 deaths per thousand live births) and rose sharply above 3500 gm. Among black twins, the lowest mortality rates were among those who weighed ≥2250 gm, with <12 deaths per thousand live births. Above 3000 gm the small numbers of black twins precluded accurate calculation of mortality rates on the basis of this matched twin pair data set. However, previous data from 1983 unpaired twin files revealed an increase in mortality above 3250

Table I. Twin infant mortality rates by birth order and gender, from Linked Birth/Infant Death Data Sets: 1983 and 1984 Birth Cohorts

	No. of twin births	*Mortality rate per 1000 live births*	*Relative risk*	*95% Confidence limits*
White twins				
Firstborn	41,554	45.3	1.0	
Second-born	41,554	48.8	1.1	1.0-1.2
Female	41,427	41.4	1.0	
Male	41,681	52.7	1.3	1.2-1.4
Black twins				
Firstborn	10,062	77.1	1.0	
Second-born	10,062	81.4	1.1	1.0-1.2
Female	10,133	73.3	1.0	
Male	9,991	85.3	1.2	1.1-1.3

Table II. Infant factors related to twin infant mortality, Linked Birth/Infant Death Data sets: 1983 and 1984 Birth Cohorts

		No. of pregnancies both died			*No. of pregnancies either died*		
Infants factors		*Infant mortality rate**	*Relative risk*	*95% Confidence limits*	*Infant mortality rate**	*Relative risk*	*95% Confidence limits*
White twins							
Gender pairs							
Unlike-gender	11,239	18.2	1.0	—	49.8	1.0	—
Female-female	15,094	30.5	1.7	1.4-2.0	60.4	1.2	1.1-1.4
Male-male	15,221	39.2	2.2	1.9-2.6	77.3	1.6	1.4-1.8
Birth weight differentials†							
A 25% >B	2,877	48.0	1.8	1.5-2.1	117.1	2.1	1.9-2.4
A 10%-25% >B	7,500	28.1	1.1	0.9-1.2	58.7	1.1	1.0-1.2
A = B	22,035	26.9	1.0	—	55.1	1.0	—
A 10%-25% <B	6,861	21.9	0.8	0.7-1.0	51.2	0.9	0.8-1.1
A 25% <B	2,039	36.8	1.4	1.1-1.7	97.2	1.7	1.4-2.0
Black twins							
Gender pair							
Unlike-gender	3,441	48.5	1.0	—	108.1	1.0	—
Female-female	3,346	46.6	1.0	0.8-1.2	97.1	0.9	0.8-1.1
Male-male	3,275	62.9	1.3	1.1-1.6	112.7	1.1	0.9-1.2
Birth weight differentials†							
A 25% >B	746	55.0	1.2	0.8-1.6	128.7	1.3	1.1-1.7
A 10%-25% >B	1,679	45.9	0.9	0.7-1.2	100.7	1.1	0.9-1.3
A = B	5,530	49.2	1.0	—	95.8	1.0	—
A 10%-25% <B	1,670	51.5	1.1	0.8-1.3	107.8	1.1	1.0-1.3
A 25% <B	403	27.3	0.6	0.3-1.0	106.7	1.1	0.8-1.5

*Per 1000 pregnancies.

†A 25% >B indicates that the difference between the twins' weight in grams (A − B) was >25% greater than the average weight of the pair; A 10% to 25% >B indicates that the difference (A − B) was 10% to 25% of the average weight of the pair; A = B indicates that the difference (A − B) was within 10% of the average weight of the pair; A 10% to 25% <B indicates that the difference (B − A) was 10% to 25% of the average weight of the pair; A 25% <B indicates that difference (B − A) was more than 25% of the average weight of the pair.

gm, indicating a similar pattern to white twins at the highest birth weights.[3]

With respect to timing of death, four fifths of white and three fourths of black twins died in the neonatal period (0 to 27 days). In the neonatal period a slightly higher percentage of infant deaths occurred among twin members of like-gender pairs than among twins of unlike-gender pairs (83% vs 78% among whites and 78% vs 71% among blacks).

Infant mortality rates by birth-order status (firstborn or second-born) and gender are presented in Table I. There were only minimal differences in mortality rates for firstborn and second-born twins. Among both races, male twins had 20% to 30% higher mortality rates than female twins.

Mortality rates with respect to twin pair characteristics are presented in Table II. Note that in this table and in Table III rates are expressed per 1000 twin

Table III. Maternal risk and twin infant mortality in United States, 1983 and 1984: Infant mortality rate per 1000 twin pregnancies

		Both twins died			*Either twin died*		
*Maternal risk level**	*No. pregnancies*	*Infant mortality rate*	*Relative risk*	*95% Confidence limits*	*Infant mortality rate*	*Relative risk*	*95% Confidence limits*
White twins							
Low	10,671	25.2	1.0	—	50.7	1.0	—
Moderate	23,447	28.0	1.1	1.0-1.3	60.9	1.2	1.1-1.3
High	1,071	53.2	2.1	1.6-2.8	103.6	2.0	1.7-2.5
Black twins							
Low	990	35.4	1.0	—	70.7	1.0	—
Moderate	6,438	42.7	1.2	0.9-1.7	96.0	1.4	1.1-1.7
High	1,637	78.2	2.2	1.5-3.2	138.7	2.0	1.5-2.6

*The low-risk group consisted of married primiparous women 20 to 29 years of age and of low parity (one prior birth to women <25 years old or two or fewer prior births to women ≥25 years old) and multiparous women who were ≥20 years old with ≥13 years of education. The high-risk group consisted of unmarried teenagers, primiparous ≥30 years old and of high parity (two or more prior births to women <25 years old or three or more prior births to women ≥25 years old), and multiparous women with <12 years of education. The moderate-risk group included all other combinations of maternal characteristics.

pregnancies, rather than per 1000 live births, in order to present the risks of both and of either (i.e., one or both) member of the twin pair dying. Among whites, like-gender pairs were at highest risk of death, compared with unlike-gender pairs, particularly for death of both twin members. White like-gender male twins had a 60% increased risk of either twin member dying and a 120% increased risk of both twin members dying, whereas white like-gender female pairs had a 20% increased risk of either twin member dying and a 70% increased risk of both members dying. Much less difference in mortality rates was noted for black like- versus unlike-gender pairs, although like-gender black male pairs did have a 30% increased risk of both members of the pair dying, compared with unlike-gender pairs. In contrast, black like-gender female pairs appeared to have a slight survival advantage, compared with unlike-gender pairs. These gender-pair mortality findings among whites may be partly related to birth weight distribution: A larger proportion of white like-gender versus unlike-gender twins were VLBW (9% vs 7%) and LBW (49% vs 43%). Among blacks, in contrast, there were almost no differences in percentages of like- versus unlike-gender VLBW twins (16% vs 16%) or LBW twins (63% vs 62%).

Assessment of infant mortality rates for either twin dying or both twin members dying in relation to birth weight differentials between the pair revealed a U-shaped curve among whites with excess mortality at the extremes (twin A >25% B or twin A <25% twin B) when compared with twin pairs whose members' birth weights were less disparate (Fig. 2). Among blacks, however, the excess mortality was evident only in the extreme at which twin A weighed more than twin B. (It is also of interest that the black/white mortality differential is considerably smaller at these extremes than in the less extreme weight difference categories.)

Twin infant mortality rates and risk ratios that are based on levels of maternal demographic risk levels (low, moderate, or high) are presented in Table III. Among whites, 30% of the twin pregnancies were to low-risk and 3% to high-risk mothers. Among blacks, 11% were to low-risk and 18% to high-risk mothers. The relative risks for infant twins born to moderate-risk mothers were 1.1 to 1.2 for whites and 1.2 to 1.4 for blacks. For high-risk mothers of both races the risk of infant death was similar: Infant twins born to white high-risk women had 2.0 to 2.1 times the risk of infant death, and twin infants born to black high-risk women had 2.0 to 2.2 times the risk of infant death, when compared with infants born to low-risk women.

Cause-specific mortality. Cause-specific mortality for firstborn and second-born twins was evaluated according to the underlying cause of death (ICD-9) recorded on the infant death certificate. The leading causes of twin mortality were neonatal deaths related to LBW. Among white twins, *Respiratory Distress Syndrome* (ICD-769) was the underlying cause for 22% of infant deaths; this, combined with *Other Respiratory Conditions of the Fetus and Newborn* (ICD-770) and *Disorders of Short Gestation and Unspecified Low Birthweight* (ICD-765), accounted for 53% of twin deaths. Among black twins, *Respiratory Distress Syndrome* was the underlying cause of death for 15% of infants, and, combined with *Other Respiratory Conditions of the Fetus and Newborn* and *Low Birthweight*, it accounted for 51% of deaths. This compares with 23% of white and 30% of black singleton deaths related to LBW and its consequences (1983 vital statistics data).[3] The infant mortality rate from these combined causes was 7.9 times higher among twins, compared with singletons.[3]

Congenital Anomalies (ICD-740-759) was the underlying cause for 10% of white and 5% of black twin infant

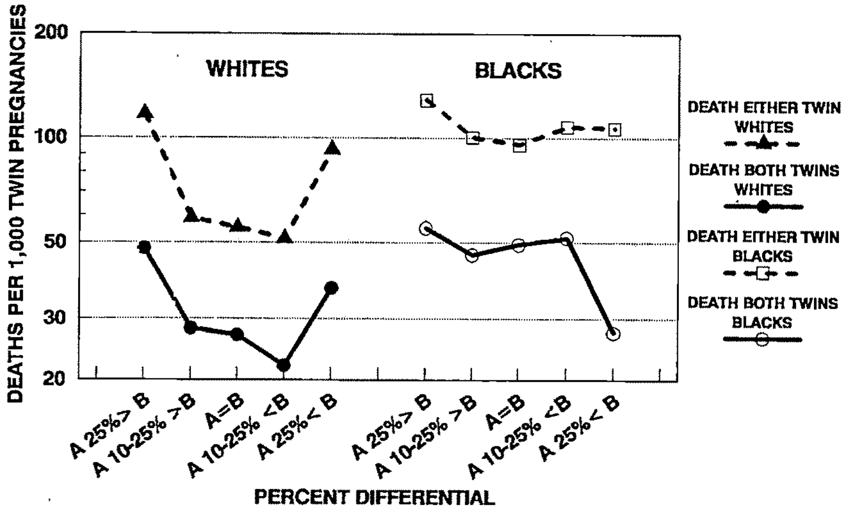

Fig. 2. Twin infant mortality rates by twin pair birth weight differential for black and white twins, from Linked Birth/Infant Death Data Sets: 1983 and 1984 Birth Cohorts for the United States.

deaths. This compares with 27% of white and 14% of black singleton deaths.[3] However, the infant mortality rate from *Congenital Anomalies* was still 1.4 times higher among twins than among singletons.[3] One third of white and 43% of black deaths from *Congenital Anomalies* were due to cardiac conditions. Among whites, deaths caused by *Congenital Anomalies* were 30% to 40% higher among like-gender twin pairs than among unlike-gender pairs.

Table IV presents mortality rates and relative risks for second-born twins compared with firstborn twins for the 10 leading causes of infant death among white twins. Although the overall mortality risk of second-born twins was only 8% greater than that of firstborn twins, mortality risks as a result of respiratory distress syndrome, asphyxia, and congenital anomalies were 19% to 27% higher among second-born twins than among firstborn twins. (Similar patterns were seen for black firstborn and second-born twins, but confidence limits were extremely wide because of the small number of cause-specific events.)

Comment

Despite overall declines in United States infant mortality since the 1950s, twin mortality continues to be four to five times that of singletons. In addition, black twin infant mortality rates are 70% higher than white twin infant mortality rates.

This study contributes to the research on twin infant mortality by using United States linked birth/death vital statistics data to clarify infant and maternal demographic risk factors associated with twin infant mortality. A major strength of the study is the use of 2 years of national data on twin infant mortality, which helps alleviate the sample size and generalizability problems that limit much of the previous research on twins. In addition, this study represents one of the few attempts to match large numbers of twin pair members to look at pair outcomes. On the other hand, a limitation is the lack of detailed data regarding maternal medical risk factors and obstetric management, since the data are limited to information recorded on birth and death certificates.

Maternal risk levels that are based on sociodemographic variables indicate a twofold increased risk for infant mortality among twins born to high-risk black or white women. Although not generally amenable to change, they do help identify particularly high-risk women carrying twin pregnancies, so that interventions to modify behavioral characteristics associated with these risk levels (e.g., smoking or poor nutritional habits) can be implemented.

The analyses of infant risk variables indicate that male, white like-gender twins and twin pairs in which the difference in weight is >25% of the average weight of the pair are at increased risk of infant mortality. Regarding the risk ascribed to birth weight differentials, Erkkola et al.[16] found similar results among a group of 460 Finnish twin pairs. These birth weight differential findings may in part reflect deleterious effects of twin-to-twin transfusion among monochorionic twins sharing vascular anastomoses or other prenatal conditions that impinge differentially on the growth of twin pair members. Ultrasonographic measurements indicating marked size differentials between twin pairs should therefore alert the clinician to higher risk of adverse outcome for either or both members of the pair.

The data regarding gender pair differences in mortality among white like-gender twin pairs support pre-

Table IV. Cause-specific infant mortality rates for white firstborn (A) and second-born (B) twins, Linked Birth/Infant Death Data sets: 1983 and 1984 Birth Cohorts*

	Death rate per 100,000 live births			
ICD category	*Twin A*	*Twin B*	*Relative risk (B/A)*	*95% Confidence limits*
All Causes	4529.4	4882.8	1.08	1.01-1.15
Respiratory Distress Syndrome	900.0	1078.3	1.19	1.04-1.37
Short Gestation/Low Birthweight	856.7	878.4	1.03	0.89-1.19
Other Respiratory Conditions of Newborn	608.8	661.8	1.09	0.92-1.29
Congenital Anomalies	404.1	502.9	1.24	1.01-1.52
Sudden Infant Death Syndrome	240.7	247.9	1.03	0.78-1.36
Maternal Complications of Pregnancy	146.8	151.6	1.03	0.72-1.46
Neonatal Hemorrhage	98.7	91.4	0.93	0.60-1.45
Circulatory System Diseases	98.5	81.7	0.83	0.53-1.31
Intrauterine Hypoxia and Birth Asphyxia	96.3	122.7	1.27	0.84-1.92
Placenta, Cord, or Membrane Complications	89.0	84.0	0.94	0.59-1.49

*ICD-761.5 *(Multiple Pregnancy)* recoded to ICD-765 *(Short Gestation/Low Birthweight)*; see text.

vious research, which found an increased risk of mortality for like-gender twins, compared with unlike-gender (dizygotic) twins.[5] A partial explanation for this finding is that the like-gender pairs include monozygotic twins, which are known to be at increased risk for placental and cord complications, respiratory distress syndrome, and congenital anomalies.[5] In addition, the larger proportion of white like-gender pairs who are LBW and VLBW, compared with unlike-gender pairs, places them at increased risk for infant mortality. The reason for the lack of protection of unlike gender (dizygosity) with respect to birth weight or survival among black twin pairs is unclear. It may reflect higher maternal complication rates (such as toxemia) among black women, which could negate vascular and nutritional benefits of two independent placental blood supplies on fetal growth. Supporting this hypothesis, Kovacs et al.[17] reported that dizygosity was associated with elevated toxemia rates, compared with monozygosity, and previous research indicates that black women have higher rates of dizygotic twinning, compared with white women. An alternative possibility is that more late fetal deaths (as opposed to early neonatal deaths) are occurring among black like-gender twin pairs, thus lowering black like-gender infant mortality rates. Because we were able to identify only twin pairs where both were live births, we could not investigate this possibility. However, it is interesting that the numbers of male black twin live births are slightly less than the numbers of female black twin live births (opposite to the expected small excess of male births), which would support the possibility of increased fetal deaths among black male twins.

Although our study found only a slight increase in overall mortality for second-born twins, these national data do reinforce previous research suggesting that second twins are at increased risk of death as a result of asphyxia[18] and newborn respiratory disease.[6]

The relative survival advantage of black infant twins in the VLBW category (Fig. 1) parallels patterns noted among black singletons in the LBW range. A potential explanation for this is that black VLBW twins tend to be more stressed in utero because of a variety of maternal sociodemographic and medical risk factors (increased rates of toxemia, poorer nutrition, young age) and that these stressors result in endogenous steroid release with greater physiologic pulmonary maturity of the infant at a given birth weight.[19] On the other hand, Wilcox and Russell[20] have suggested that race-specific birth weight standards should be used when infant mortality in blacks and whites is compared, since the optimal birth weight for survival is markedly lower in blacks. According to their model, if one uses a standardized scale to align the black and white birth weight distributions, then the relative survival advantage of lower birth weight black infants disappears. In any case the survival disadvantage of black twins of normal birth weight is disturbing and suggests that preventable deaths may be occurring.

The extremely high infant mortality seen among both white and black twins probably reflects (1) an accentuation of maternal medical complications in the presence of background sociodemographic risk factors and (2) infant risk factors associated with twinning. Since much of the infant mortality among twins is associated with consequences of very low birth weight, an obvious challenge is to develop strategies that decrease the proportion of twins born at the skewed lower tail of the twin birth weight distribution. Successful interventions to decrease infant mortality among twins should be applicable to other high-risk groups because twins can be viewed as a model demonstrating both demographic and medical risk factors associated with infant mortality. The double jeopardy of both maternal and infant twin risk factors suggests the need for multifaceted approaches to achieve this goal.

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Twin delivery: Influence of the presentation and method of delivery on the second twin

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To ascertain the perinatal mortality and morbidity in the second twin as related to its presentation and method of delivery, we reviewed the data on 578 sets of twins delivered from 1980 to 1987 and included 397 sets in whom both twins were ≥1000 gm, without lethal anomalies, and in whom the first twin presented as a vertex. The perinatal outcome comparing twin A (all vertex) with twin B (vertex or nonvertex) with cesarean section or vaginal delivery was analyzed. No statistically significant difference in perinatal mortality or morbidity was found in comparing the nonvertex second twin delivered vaginally or by cesarean section. The one perinatal death and significant perinatal morbidity occurred in infants weighing <1500 gm or at <32 weeks' gestational age. It is concluded that vaginal delivery, irrespective of the position of the second twin, is valid in selected cases as long as fetal weight is >1500 gm and the gestational age is ≥32 weeks. (AM J OBSTET GYNECOL 1991;165:23-7.)

Key words: Twin delivery, perinatal outcome

Controversy continues over the better route of delivery of twins, especially when one or both fetuses are not in cephalic presentation. Cetrulo[1] advocates a liberal cesarean section policy for nonvertex second twins (84% for vertex-nonvertex presentations >34 weeks' gestation) and with this practice has obtained similar mortality and morbidity for each member of the twin pair. Other reports[2-8] document no increase in morbidity and mortality in nonvertex second twins weighing >1500 gm, irrespective of the route of delivery. Doyle et al.[9] assessed twin sets <32 weeks' gestation and reported a 9.6% cesarean section rate for vertex-vertex and vertex-nonvertex presentations combined. They found no difference in mortality or immediate morbidity between vaginal and cesarean delivery.

Slowly rising cesarean section rates from 25% to 40% for twins during the 6-year period 1980 to 1985 in our hospital prompted us to review twin delivery and the associated mortality and morbidity.

Material and methods

From our perinatal data base we reviewed all twin deliveries (578 sets) during the 8-year period 1980 to 1987. The following inclusion criteria were fulfilled by 397 of the original twin sets: (1) twin A in vertex presentation; (2) lethal anomalies excluded; and (3) each twin ≥1000 gm.

All cases with the following mortality and morbidity were categorized with the use of information in the perinatal data base: stillbirths and neonatal deaths ≤28 days; grade III and IV intraventricular hemorrhages (on the basis of ultrasonography); postasphyctic or posttraumatic convulsions; moderate and severe respiratory distress syndrome (on the basis of clinical and radiologic signs); Apgar score <7 at 5 minutes; and trauma (defined as fractures or nerve palsies).

All charts were reviewed to determine the presentation in labor, intrapartum manipulations, mode of delivery, and neonatal outcome. The χ^2 and Fisher's exact test were used to calculate statistical significance when comparing two groups.

Results

Ten stillbirths (all antepartum) were identified in seven twin sets. There was one neonatal death in a second twin delivered vaginally in breech presentation at 31 weeks' gestation. The infant weighed 1880 gm and died of severe hydrops as a result of twin-to-twin transfusion. The perinatal mortality for twins ≥1000 gm and without lethal anomalies was 13.8/1000 or 1.3/1000 for those alive at the onset of labor. This compares with an overall perinatal mortality of 82/1000 for all twins ≥500 gm born from 1980 to 1987.

Once twin sets with antenatal stillbirths (seven) and nonlabor cesarean sections (25) are excluded, the presentation of 365 twin pairs in the early phase of labor and their subsequent presentation and mode of delivery are shown in Table I.

Table II lists the indications for cesarean section in-

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Table I. Relationship of twin presentation in early labor to the presentation and mode of delivery

Presentation in early labor			*Vaginal delivery*		*Cesarean section*	*Twin A, vaginal; twin B, cesarean section*
	No.	*%*	*Vertex-vertex*	*Vertex-breech*		
Vertex-vertex	146	40	117	4	20	5
Vertex-transverse	30	8	6	17	4	3
Vertex-breech	90	25	5	69	15	1
Vertex-unrecorded	99	27	68	26	3	2
TOTAL	365	100	196 (54%)	116 (32%)	42 (11%)	11 (3%)

Table II. Cesarean section: Indications (79 twin sets)

Elective (n = 25)			*In labor (n = 43)*			*For twin B only (n = 11)*		
Indication	*No.*	*(%)*	*Indication*	*No.*	*%*	*Indication*	*No.*	*%*
Previous cesarean section	21	84	Dystocia	35	81	Uncorrectable transverse lie	5	46
Hypertension	2	8	Fetal distress	3	7	Failure to descend	3	27
IUGR	1	4	Abruptio placentae	3	7	Brow presentation	2	18
Other	1	4	Other	2	5	Cord prolapse	1	9

IUGR, Intrauterine growth retardation.

volving 79 of the 397 twin sets (20%). In 11 cases, the second twin was delivered by cesarean section after vaginal delivery of the first twin.

Tables III and IV present the perinatal morbidity related to birth weight: 1000 to 1499 gm and ≥1500 gm, respectively. The two main columns identify twin pairs delivered as vertex-vertex or vertex-nonvertex (nonvertex = breech or transverse presentations). These groups are each subdivided into cesarean or vaginal delivery. The numbers include only those infants alive at the start of labor. The only complication not shown in the tables is trauma. There was one case—a fractured humerus in a second twin weighing 3300 gm (600 gm heavier than its companion vertex delivery) and described as a difficult assisted breech delivery.

Table III shows the outcome of infants weighing between 1000 and 1499 gm. Three intraventricular hemorrhages occurred. Both members of one vertex-nonvertex twin pair delivered vaginally had grade III intraventricular hemorrhages. The gestational age was 27 weeks and the deliveries were atraumatic with normal cord pH values. (Twin B was delivered by breech extraction after successful internal podalic version.) Both infants had severe respiratory distress syndrome (RDS). The other intraventricular hemorrhage was associated with difficult breech extraction of the second twin after internal podalic version at 28 weeks' gestation. This infant also had moderate RDS and convulsions. There is no statistical difference in morbidity between vaginal breech delivery and cesarean section in infants 1000 to 1499 gm.

Table IV reviews twins ≥1500 gm. One intraventricular hemorrhage occurred in a second twin and this was associated with an uncomplicated breech extraction at 31 weeks' gestation of an infant weighing 1905 gm. Forceps were applied to the aftercoming head and the cord pH value was normal. The intraventricular hemorrhage in twin A was detected on day 9 when the infant started convulsing and was believed to be related to an anteriovenous malformation and not to birth trauma. The only other case of convulsions was associated with cord prolapse in a first twin subsequently delivered by cesarean section with Apgar scores of 1 and 4 at 1 and 5 minutes, respectively. No statistically significant difference in morbidity was observed between the groups.

Table V analyzes the methods of delivery and manipulation of the second twin in breech and transverse presentations and their relationship to intraventricular hemorrhage. Twenty-nine internal podalic versions were attempted: five failed and twin B was delivered by cesarean section without complications. Twenty-four infants were delivered vaginally after successful internal podalic version and two of these had intraventricular hemorrhage. Both of these infants weighed <1500 gm and were <29 weeks' gestation. Of 41 breech extractions performed, one intraventricular hemorrhage occurred in an infant weighing 1905 gm at 31 weeks' gestation. The extraction was described as uncomplicated and the Apgar scores were 7 and 8 at 1 and 5 minutes, respectively. Of all nonvertex second twins who were delivered vaginally, 13 of the 17 infants (76%)

Table III. Twin delivery: Perinatal morbidity 1000 to 1499 gm (58 infants)

	Vertex-vertex				Vertex-nonvertex			
	Cesarean section		Vaginal		Cesarean section		Vaginal	
	A	B	A	B	A	B	A	B
Total deliveries	0	3	10	10	2	3	13	17
Convulsions	—	—	—	—	—	—	—	1
IVH	—	—	—	—	—	—	1	2
RDS	—	—	—	3	1	1	3	7
Apgar score <7	—	—	—	2	—	—	2	6

IVH, Grade III or IV intraventricular hemorrhage.

Table IV. Twin delivery: Perinatal morbidity ≥1500 gm (726 infants)

	Vertex-vertex				Vertex-nonvertex			
	Cesarean section		Vaginal		Cesarean section		Vaginal	
	A	B	A	B	A	B	A	B
Total deliveries	38	40	195	186	28	32	108	99
Convulsions	1	—	1	—	—	—	—	—
IVH	—	—	1	—	—	—	—	1
RDS	—	—	1	4	1	1	5	6
Apgar score <7	1	—	1	4	1	2	—	3

IVH, Grade III or IV intraventricular hemorrhage.

weighing 1000 to 1499 gm at birth had internal podalic version or breech extraction performed compared with 52 of the 99 infants (52%) with birth weights ≥1500 gm.

As illustrated in Table I, in 218 twin sets delivered vaginally the presentation of both twins was documented in early labor and again after delivery of the first twin. In 121 vertex-vertex combinations, six (4.9%) second twins spontaneously turned to a nonvertex presentation after delivery of the first twin (three had successful external cephalic version, two had internal podalic version and breech extraction, and one had an assisted breech delivery). In addition, one second twin was turned from vertex to breech by internal podalic version and was delivered by breech extraction. In 74 vertex-breech combinations, six (8.1%) second twins spontaneously turned to vertex (two) or transverse (four) presentations. In 23 vertex-transverse combinations, nine (39%) second twins turned to vertex (two) or breech (seven) presentations after delivery of the first twin. There were 15 documented attempts at external cephalic version of the second twin with success in 12 followed by delivery in vertex presentation. Of the three failed external cephalic versions, one was turned to breech and the remaining two had internal podalic version and breech extraction without morbidity.

Table VI illustrates the delivery interval between twins and the mode of delivery and resultant morbidity of the second twin; 90% of vertex and 97% of nonvertex second twins were delivered within 30 minutes of the first twin. The longest delivery interval for a vertex twin was 277 minutes compared with 64 minutes for a nonvertex twin. There was no significant difference in low Apgar scores between delivery intervals <15 minutes and those between 15 and 30 minutes. All three intraventricular hemorrhages occurred in twins delivered within 15 minutes of their companion twin. Prolongation of the delivery interval >30 minutes did not add to morbidity, but overall numbers are low in this group. Cesarean section was performed for three vertex twins after intervals >1 hour (69, 78, and 277 minutes).

Comment

One of the main areas of controversy in twin pregnancies is the delivery of vertex-nonvertex presentations. We are aware of only one small prospective randomized study[3] that is available and therefore one has to rely on local experience and nonrandomized studies to formulate a policy for delivery.

In our review of vertex-vertex and vertex-nonvertex twin sets without lethal anomalies and with both twins ≥1000 gm, we recorded an 80% vaginal delivery rate. Perinatal mortality was not influenced by vaginal breech delivery of the second twin. However, we realize

Table V. Twin B nonvertex: Relationship of method of delivery to intraventricular hemorrhage

	1000-1499 gm		*≥1500 gm*		*Total*	
	IVH	*No. of infants*	*IVH*	*No. of infants*	*IVH*	*No. of infants*
Assisted breech (n = 51)	0	4	0	47	0	51
Breech extraction (n = 41)	0	7	1	34	1	41
Internal podalic version + breech extraction (n = 24)	2	6	0	18	2	24

IVH, Intraventricular hemorrhage.

Table VI. Relationship of delivery interval between twins and mode of delivery and morbidity of twin B (excludes cases with both twins delivered by cesarean section)

	Delivery interval (min)		
	<15	*16-30*	*>30*
Cephalic			
Spontaneous	66	13	3
Forceps	74	26	14
Breech			
Assisted	44	4	3
Extraction	61	3	1
Cesarean section (twin A vaginal)	0	8	3
TOTAL	245 (76%)	54 (17%)	24 (7%)
External cephalic version	6	2	4
Internal podalic version	21	2	1
5 min Apgar score <7	14	2	0
Intraventricular hemorrhage	3	0	0

that because of our low numbers and the paucity of adverse effects the possibility of a β-error could exist. In reviewing morbidity rates, the presence of three significant intraventricular hemorrhages in nonvertex second twins delivered vaginally raised concern. Internal podalic version and breech extraction were performed in the two second twins weighing <1500 gm with intraventricular hemorrhage. In one twin set in whom both members had grade III intraventricular hemorrhage and severe RDS, it is difficult to implicate the version and delivery as the probable cause of hemorrhage. However, the other case was characterized as being a difficult delivery of both first and second twins, resulting in low Apgar scores in each and an intraventricular hemorrhage in twin B. In twins weighing >1500 gm, one intraventricular hemorrhage was associated with an uncomplicated breech extraction with forceps applied to the aftercoming head. This infant also had moderate RDS that may have been a contributing factor.

Three fourths of the internal podalic versions were performed in twins weighing >1500 gm and no significant neonatal morbidity was found in this group. In analyzing these results, we believe that this procedure is safe in twins weighing >1500 gm. This is in agreement with reports from Rabinovici[10] and Chervenak.[2,6] We realize that this subgroup of patients may have been delivered by operators comfortable with and experienced in internal podalic versions, hence giving acceptable results that may not be reproducible with less experienced physicians. However, early recourse to cesarean section was undertaken in those cases of attempted version that proved difficult. By so doing, there were no cases of major morbidity in our cesarean sections performed for the second twin alone. In spite of our low numbers this indicates that this procedure seems to be a safe alternative to vaginal delivery and should be considered if difficulty is encountered in delivery of the second twin.

Breech extraction alone was performed in 41 second-twin deliveries with one case of intraventricular hemorrhage. When combined with internal podalic version and breech extraction, a total of 65 deliveries are documented. Fifty-two of these (80%) occurred in twins weighing >1500 gm, and only one intraventricular hemorrhage was encountered. Unfortunately, it is difficult to compare this group with second twins delivered by spontaneous breech delivery or cesarean section because of low numbers and the rarity of poor outcome. In this retrospective review we do not know why some breech presentations were delivered by assisted breech delivery and others by breech extraction. Indications for prompt delivery (e.g., fetal distress) along with operator bias and experience were probably the main reasons. We do not advocate routine breech extraction but rather our results support the work of others that show selected breech extraction to be an acceptable method of delivery in twins >1500 gm.[2,3,5-8]

Twelve of 15 documented external cephalic versions performed in this group were successful. Of the three failures, all had uneventful vaginal breech deliveries. Although our numbers are very small, this compares favorably with Chervenak's[11] report of a 72% success rate, whereas not showing the complications encountered by Gocke et al.[12] in their study of 41 versions. Further studies are needed to show whether this procedure is as safe as originally thought and its role as an alternative to cesarean section of the nonvertex second twin <1500 gm should be further explored.

Our experience is in agreement with that of Rayburn et al.[8] in that as long as fetal surveillance is maintained, there need be no rigid time restriction for delivery interval between twins. We advocate conservative management and oxytocin augmentation for delay with both vertex and frank or complete breech presentations. Should the second twin present as a footling breech then breech extraction can be considered. If a transverse lie is encountered we favor external cephalic version. Should this fail, internal podalic version and breech extraction should be carried out. If the operator is uncomfortable with this procedure early recourse to cesarean section is an alternative.

We conclude therefore that it is reasonable to allow labor and vaginal delivery, irrespective of the presentation of the second twin, as long as fetal weight is ≥1500 gm and the gestational age ≥32 weeks. This accounts for >85% of vertex-nonvertex twin pairs (≥500 gm) entering labor in our series. In smaller twins, a dilemma is posed: the numbers are low, especially involving twins delivered by cesarean section, and yet there were two cases of major morbidity associated with vaginal delivery that involved internal podalic version and breech extraction in second twins weighing between 1000 and 1499 gm. Although no statistical differences were seen, we believe that internal podalic version and breech extraction should be avoided if possible in this group, and that external version or cesarean section may be associated with less major morbidity.

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Cigarette smoking during pregnancy in relation to placenta previa

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To evaluate the relationship between cigarette smoking and the occurrence of placenta previa, we used interview and medical record data to conduct a case-control analysis of 69 placenta previa cases and 12,351 controls. The unadjusted relative risk estimate of placenta previa for women reported to have "ever smoked" during pregnancy relative to nonsmoking mothers was 1.9 (95% confidence interval, 1.2 to 3.0). The risk rose after adjusting for potential confounders (odds ratio, 2.6; 95% confidence interval, 1.3 to 5.5). In contrast to a previous report, the duration of smoking was not an independent risk factor for placenta previa. These results suggest that cigarette smoking during pregnancy is a determinant of placenta previa. Carbon monoxide hypoxemia, which is one possible mechanism for this association, may result in compensatory placental hypertrophy. Placentas with increased surface areas are more likely to cover the cervical os, causing placenta previa. (AM J OBSTET GYNECOL 1991;165:28-32.)

Key words: Placenta previa, pregnancy, smoking, risk factor

Although placenta previa is uncommon, it is a significant cause of maternal and perinatal morbidity and mortality. Placenta previa has been reported to occur in 3.3 to 9.9 per 1000 deliveries.[1, 2] However, its cause is poorly understood. Risk of placenta previa has been found to increase with increasing parity, maternal age, previous induced and spontaneous abortions, previous cesarean section, a history of dilatation and curettage, and short maternal stature; it also occurs more frequently with singleton male pregnancies and twin pregnancies of both sexes and when gestation takes place at high altitude.[1-10]

Two prior studies found a positive association between placenta previa and maternal cigarette smoking during pregnancy.[5, 11] Meyer et al.[5] observed a 25% and 92% increase in the prevalence of placenta previa for smokers of less than one pack and smokers of one or more packs of cigarettes per day, respectively. More recently, Kramer et al.[11] also found an association between smoking and placenta previa (odds ratio, 1.9; 95% confidence interval, 1.5 to 2.8).

Naeye[12] reported that the duration of maternal cigarette smoking was associated with placenta previa. He found that the frequency of placenta previa was more strongly associated with the number of years mothers had smoked than with their smoking habits during pregnancy. In this study we evaluated the association between cigarette smoking and placenta previa in a large cross-sectional data base.

Material and methods

The Delivery Interview Program, conducted at the Boston Hospital for Women from August 1977 to March 1980, was designed to study the occurrence of adverse outcomes of pregnancy.[13] Information was obtained by personal interviews and medical records review. During the study period 14,458 mothers (84.4% of all deliveries) were asked to participate in the study. Some mothers were not asked to participate because there were too few interviewers (14.1%) or because their physicians would not grant permission for participation in the study (1.4%). When too few interviewers were available, patients were selected randomly. Of the mothers with singleton deliveries, 90.0% of those approached (12,825 women) were interviewed. The reasons for not having been interviewed were early discharge (5.5%), refusal to be interviewed (3.0%), the presence of a language barrier (1.6%), and medical conditions precluding an interview (0.1%). The interview provided information on demographic character-

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Table I. Labor and delivery characteristics of women with placenta previa (cases) and control subjects

Variable	*Placenta previa cases (N = 69)*	*Control subjects (N = 12,351)*
Cesarean section (%)	82.6	21.3
Birth weight (gm)*	2645 ± 798	3316 ± 675
Weeks' gestation*	36.1 ± 4.2	39.7 ± 2.5
Neonatal deaths/1000 deliveries (deaths in first 7 days of life)	14.5	3.7
Perinatal deaths/1000 deliveries†	14.5	9.1

*Mean ± SD.
†Stillbirths and neonatal deaths.

istics, previous contraceptive practice, medical and reproductive histories, cigarette smoking habits, and exposure to other possible risk factors. Information regarding the duration of smoking; smoking status at the time of conception; and the average number of cigarettes smoked per day during the first, second, and third trimesters, respectively, was obtained from mothers who had ever smoked. Information was collected from the medical records regardless of whether an interview had occurred. Information regarding pregnancy outcome and condition of the newborn was abstracted from the mothers' and infants' medical charts. Completed interviews and medical records information were available for 12,718 women who were delivered of singleton infants.

For this analysis, cases consisted of nondiabetic women with singleton deliveries whose medical records indicated a physician's diagnosis of placenta previa ($n = 69$). Detailed information regarding gestational age at diagnosis and type of placenta previa (marginal, partial, or total) were not available. The controls were the 12,351 nondiabetic women with singleton deliveries that were not complicated by placenta previa. This study was restricted to nondiabetic women because only 2.3% of the study population were diabetic. Women with a diagnosis of abruptio placentae (independent of placenta previa) indicated on their medical records ($n = 135$) were excluded from this analysis. Abruptio placentae, another cause of third-trimester bleeding, has been shown to be associated with maternal cigarette smoking during pregnancy.[5] Analyses including these women yielded results similar to those reported here.

Women were considered to be cigarette smokers during pregnancy if they had reported ever having smoked during the first or second trimester. Three levels of cigarette smoking during pregnancy were considered: nonsmokers, smokers of 1 to 9 cigarettes per day, and smokers of ≥10 cigarettes per day. Three levels of the duration of cigarette smoking were also considered: never smokers, smokers for 1 to 5 years, and smokers for ≥6 years.

To estimate the risk of placenta previa associated with cigarette smoking, odds ratios were calculated. Unconditional logistic regression was used to adjust for potential confounding variables (included as either categorical or continuous variables) and to derive maximum likelihood estimates of combined relative risks and 95% confidence intervals.[14] Adjusted dose-response relationships between cigarette smoking and placenta previa were evaluated in multiple logistic regression models, with cigarette smoking treated either as a continuous variable or as a series of dichotomous variables. Level of statistical significance was fixed at $p = 0.05$. We assessed confounding by entering potential confounders into a logistic regression model one at a time and by comparing the adjusted and unadjusted odds ratios.[14] The final logistic regression model included variables that had altered the unad-

Table II. Distribution of placenta previa cases and control subjects by selected risk factors and cigarette smoking status

Variable	*Placenta previa cases (N = 69) (%)*	*Control subjects (N = 12,351) (%)*
Age >34 yr*	17.4	8.7
Black	7.2	15.4
Medicaid recipient	13.0	16.0
Marital status single	8.7	15.9
Parity*		
0-1	26.1	50.8
2-4	65.2	46.7
≥5	8.7	2.5
Spontaneous abortion*		
0	60.9	83.0
1	24.6	13.1
≥2	14.5	3.9
Prior induced abortion	10.1	14.7
Prior cesarean section*	17.4	8.1
Prior uterine surgery	2.9	0.9
DES in utero*	5.8	1.6
Maternal height <60 in	4.4	2.5
Male infant	50.7	51.9
Marijuana smoker	4.4	10.1
Alcohol ≥3 drinks/wk	10.1	8.9
Coffee ≥3 cups/day*	18.8	10.4
Current smoker*	44.9	30.3

*Two-sided $p < 0.05$.

Table III. Frequency of cases and controls, crude and adjusted odds ratios, and 95% confidence intervals for smoking and placenta previa*

Variable	*Placenta previa cases (N = 69) (No.)*	*Control subjects (N = 12,351) (No.)*	*Crude odds ratio*	*Adjusted odds ratio*	*Adjusted 95% confidence interval*
Smoker during pregnancy					
No	38	8606	1.0	1.0	–
Yes	31	3745	1.9	2.6	1.3-5.5
Cigarettes smoked per day					
Nonsmoker	38	8606	1.0	1.0	–
1-9	14	1507	2.1	3.1	1.4-6.6
≥10	17	2238	1.7	2.2	0.9-5.1
Smoking history					
Never smoker	25	5704	1.0	1.0	–
Former smoker					
Stopped before conception	13	2722	1.1	1.3	0.5-3.3
Stopped during first trimester	4	816	1.1	1.9	0.6-6.7
Smoked throughout pregnancy	27	3109	2.0	3.1	1.2-8.1

*Adjusted for race, maternal age, payment status, marital status, parity, previous spontaneous abortions, previous cesarean section, previous in utero exposure to DES, coffee consumption, and duration of cigarette smoking.

justed odds ratio by at least 10%. Variables with a priori interest also were added to the final model.

Results

The prevalence of placenta previa at the Boston Hospital for Women from 1977 to 1980 was 5.5 per 1000 deliveries among nondiabetic women. Selected delivery and infant outcomes are presented in Table I. Cases were approximately four times more likely to have been delivered by cesarean section than were controls. The mean length of gestation was 36.1 weeks for cases and 39.7 weeks for controls. Some sociodemographic, medical, and behavioral characteristics of cases and controls are presented in Table II. The mean age of cases was 29.9 years as compared with 27.7 years for controls. Cases were of higher parity than were controls. Twenty-seven cases (39.1%) and 2098 controls (17.0%) had a history of one or more spontaneous abortions. Cases were twice as likely to have had a previous cesarean section than were controls. Cases also reported a history of in utero exposure to diethylstilbestrol (DES) more frequently than did controls. Advanced maternal age, higher parity, previous in utero exposure to DES, previous cesarean section, previous spontaneous abortion, and consumption of an average of three or more cups of coffee per day during pregnancy were all statistically significant risk factors for placenta previa in this population.

A higher proportion of cases (44.9%) than controls (30.3%) reported smoking during pregnancy. Smokers for a duration of ≥6 years were more likely to have continued smoking during pregnancy (68.4%) than were smokers of 1 to 5 years (31.6%) (not shown in tables). Women who smoked during the first two trimesters of pregnancy had a 90% increase in risk for placenta previa (odds ratio, 1.9; 95% confidence interval, 1.2 to 3.0) compared with women who did not smoke during pregnancy. Controlling for potential confounders (race, maternal age, payment status, marital status, parity, previous spontaneous abortions, previous cesarean section, previous in utero exposure to DES, coffee consumption during pregnancy, and the duration of cigarette smoking) resulted in a relative risk estimate of 2.6 (95% confidence interval, 1.3, 5.5 Table III). Among smokers a dose-response gradient between placenta previa and the number of cigarettes smoked per day during pregnancy was not present when the number of cigarettes smoked during pregnancy was represented as a continuous variable (p value for linear trend, 0.75).

Compared with never-smokers, women who smoked throughout pregnancy had a threefold increase in risk for placenta previa (adjusted odds ratio, 3.1; 95% confidence interval, 1.2 to 8.1). For women who stopped smoking before conception (as compared with never smokers), the adjusted odds ratio was 1.3 (95% confidence interval, 0.5 to 3.3). For women who stopped smoking during the first trimester (as compared with never smokers), the adjusted risk was 1.9 (95% confidence interval, 0.6 to 6.7). Among former smokers estimates of the relative risk for placenta previa did not vary as a function of the duration of cigarette smoking (data not shown). The small number of women in these categories, however, necessitates caution in interpretation.

Crude and adjusted estimates of relative risk for women in each of the duration of cigarette smoking categories as compared with never smokers are shown in Table IV. The duration of cigarette smoking was not found to be an independent risk factor for placenta previa when smoking during pregnancy was considered. Women who reported smoking for 1 to 5 years

Table IV. Frequency of cases and controls, crude and adjusted odds ratios, and 95% confidence intervals for duration of smoking and placenta previa*

Duration of cigarette smoking	*Placenta previa cases (N = 69) (No.)*	*Control subjects (N = 12,351) (No.)*	*Crude odds ratio*	*Adjusted odds ratio*	*Adjusted 95% confidence interval*
Never smoker	25	5704	1.0	1.0	–
Smoker of 1-5 yr	11	2726	0.9	0.9	0.4-2.0
Smoker of ≥ 6 yr	33	3921	1.9	1.0	0.5-2.2

*Adjusted for maternal cigarette smoking status during pregnancy, race, maternal age, payment status, marital status, parity, previous spontaneous abortions, previous cesarean section, previous in utero exposure to DES, and coffee consumption.

did not have an increase in risk for placenta previa (adjusted odds ratio, 0.9; 95% confidence interval, 0.4 to 2.0). Similarly, women who had smoked for ≥6 years were not at increased risk for placenta previa as compared with never smokers (adjusted odds ratio, 1.0; 95% confidence interval, 0.5 to 2.2). A continuous variable representing the duration of cigarette smoking in years was not statistically significant in a logistic regression model confined to ever smokers ($p = 0.39$ for linear trend).

Adjusted odds ratios for maternal cigarette smoking during pregnancy and other variables entered into the final logistic regression model are presented in Table V. In addition to maternal cigarette smoking during pregnancy, prior spontaneous abortions, prior cesarean section, prior exposure to DES in utero, and increasing parity were independent and statistically significant risk factors for placenta previa.

Comment

Using cross-sectional data collected from a largely white obstetric population, we found the crude relative risk of placenta previa to be 1.9 (95% confidence interval, 1.2 to 3.0) among women reported to have ever smoked during pregnancy. Adjusting for potential confounders enhanced the observed positive association between cigarette smoking and placenta previa (adjusted odds ratio, 2.6; 95% confidence interval, 1.3 to 5.5). A dose response between the number of cigarettes smoked per day during pregnancy and the risk for placenta previa was not demonstrated.

The statistically significant increased risk of placenta previa associated with increasing parity and previous spontaneous abortion demonstrated in this report is consistent with other studies.[2, 3, 5, 6, 15] Results from two previous investigations support the hypothesis that cigarette smoking during pregnancy is an independent risk factor for placenta previa.[5, 11] Kramer et al.[11] noted a 90% increase in risk of placenta previa among women who smoked as compared with nonsmokers (adjusted odds ratio, 1.9; 95% confidence interval, 1.5 to 2.8).

The duration of cigarette smoking was not found to be an independent risk factor for placenta previa. This finding differs from that reported by Naeye.[12] In our study smoking for a duration of ≥6 years was associated with placenta previa in the initial univariate analyses. However, when cigarette smoking during pregnancy and confounders were added to a logistic regression model, the association between the duration of cigarette smoking and placenta previa was not statistically significant.

It is unlikely that bias is responsible for the associations observed in this study. There is no indication that the observed association between placenta previa and cigarette smoking was due to information bias because the trained interviewers were kept unaware of the study objective. A systematic selection bias seems unlikely because women were included in the analysis without reference to their exposure or outcome status. Some recall bias on the part of the placenta previa cases is possible and could have resulted in an overestimation of the strength of the positive association observed in this study. Nondifferential misclassification of the number of cigarettes smoked during pregnancy would tend to result in an underestimation of the strength of this association[16] between cigarette smoking and placenta previa.

Table V. Adjusted* odds ratios of placenta previa and 95% confidence intervals according to selected characteristics

Characteristic	*Adjusted odds ratio*	*Adjusted 95% confidence interval*
DES in utero	4.4	1.6-12.4
Current cigarette smoker	2.6	1.3-5.5
Prior cesarean section	1.8	1.0-3.5
Prior spontaneous abortions (No.)	1.4	1.1-1.8
Coffee ≥ 3 cups/day	1.3	0.7-2.5
Parity (No.)	1.3	1.1-1.6
Duration of cigarette smoking (yr)	1.0	0.9-1.0
Maternal age (yr)	1.0	1.0-1.1
Medicaid recipient	0.9	0.4-2.3
Single	0.7	0.2-1.9
Black	0.5	0.2-1.3

*Adjusted with the use of a multiple logistic regression model.

The observed association between cigarette smoking and placenta previa has compelling biologic plausibility. Hypoxemia as a result of exposure to nicotine and carbon monoxide from maternal cigarette smoking during pregnancy may result in compensatory placental hypertrophy, thus increasing the likelihood of encroachment of the placenta on the cervical os. Investigators have demonstrated an acute decrease in uterine blood flow immediately after maternal infusion of nicotine,[17-19] and nicotine, which is a very potent vasoconstrictor, has been shown to concentrate in the placental tissue of vertebrates.[20, 21]

Three studies that compared the placentas of smokers and nonsmokers demonstrated a 0.69% to 3.63% increase in placental weight among smokers as compared with nonsmokers.[22-24] One of these studies noted that increased placental weight was accompanied by a 1.85% to 2.37% decrease in placental thickness and by a 0.35% to 1.30% increase in the smallest placental diameter in the placentas of smokers as compared with those of nonsmokers.[24] The study findings suggest that, in comparison with nonsmokers, women who smoke during pregnancy have heavier, thinner placentas with larger surface areas; these features could result in an increased likelihood of placental encroachment on the cervical os.

Investigators have found both light microscopic and electron microscopic abnormalities in the placentas of smokers, such as fibrinoid changes in the maternal decidual arteries, fibrotic peripheral villi, villous hyperplasia, marginal placental necrosis, and hypotrophic and avascular stem villi, which they have considered to be evidence of chronic hypoxemia and ischemia.[17, 20, 25] In addition, the number of placental infarcts was found to be increased in smokers.[12] Notably, similar morphologic changes have been observed in placentas from gestations that occurred in the relatively hypoxic environment of high altitude.[9] Furthermore, McClung[9] has reported a higher prevalence of placenta previa at high altitude than at sea level.

Both laboratory and epidemiologic studies provide evidence of an association between placenta previa and cigarette smoking that is biologically plausible. The striking similarity of morphologic changes in placentas observed at high altitude and in the placentas of smokers supports the hypothesis that hypoxemia may be an important determinant in the occurrence of placenta previa.

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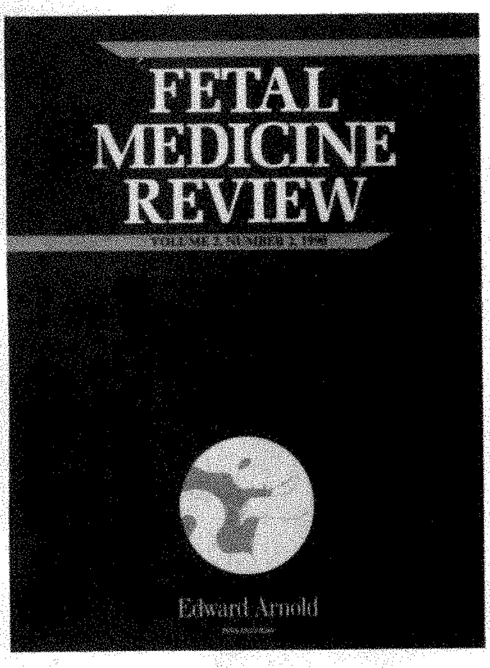

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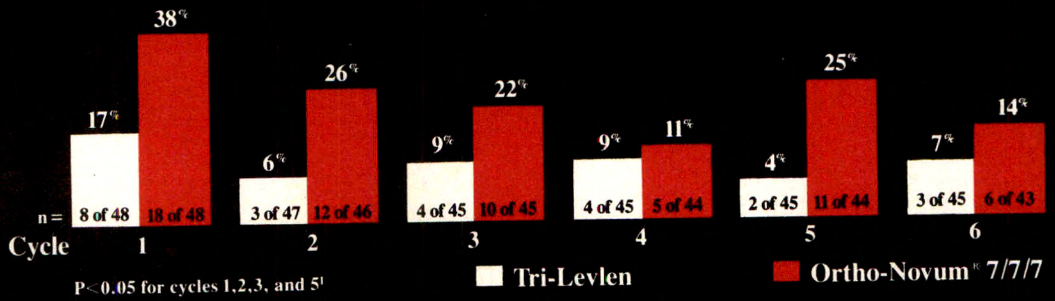

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Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction*. An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors.

b. *Thromboembolism*. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women witb predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases*. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives*. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease*. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use*. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's–but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs*. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia*. Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions*. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy*. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed.

7. *Gallbladder Disease*. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens.

8. *Carbohydrate and Lipid Metabolic Effects*. Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure*. An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache*. The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities*. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child.

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea; vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert. 60627-0 5/90

Reference: 1. Data on file, Berlex Laboratories.

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Medication in early pregnancy: Prevalence of use and relationship to maternal characteristics

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Medication use was assessed in a population of 4186 women who were delivered of infants at Yale–New Haven Hospital, New Haven, Connecticut, between 1980 and 1982. The frequency of over-the-counter and prescription drug use, and the association of such use with maternal characteristics was studied. Of all women, 66% used at least one drug. The mean number of drugs used by all subjects was 1.3 and, among drug users, 2.9. Of all drugs used, 68% were over-the-counter and 32% were prescription preparations. Internal analgesics, autonomic drugs, antiinfective agents, and antacids were most frequently used. Women who used prescription drugs were also more likely to use over-the-counter medications. Maternal characteristics that were associated with an increase in both over-the-counter and prescription drug use were being white, smoking more than 20 cigarettes a day, using alcohol, using caffeine, and smoking marijuana. These findings suggest that women who engage in "risk-taking behavior" during pregnancy are also more likely to use medications while pregnant. (AM J OBSTET GYNECOL 1991;165:33-40.)

Key words: Drugs, medications, risk-taking behavior, pregnancy

During the past 25 years there has been increasing awareness of the potentially harmful effects of drugs and chemicals on the developing fetus. However, the mechanism of most teratogenic compounds is still poorly understood and very little information exists concerning the absolute safety of the use of specific medications during pregnancy.

Knowledge of the potential danger of medication use during pregnancy and recognition of the fact that important information is still lacking should warrant conservative use of medications. Unfortunately, medication use during pregnancy is quite prevalent and does not appear to be decreasing. During the past 25 years, several studies of the prevalence of drug use during pregnancy have been carried out.[1-6] Although the studies differ in several aspects (patient population, year of study, type of drugs studied, size of study), they all report a large number of drugs being used by pregnant women: 44% to 92% of women using at least one medication, with an average of 3 to 5.5 medications per woman among users. There also does not appear to be a downward trend in frequency of use in more recent studies.

Table I. Prevalence of prescription and nonprescription drug use in pregnancy among women delivered of infants at Yale–New Haven Hospital, 1980 to 1982

No. of drugs	*Frequency*	%
0	1425	34.1
1	1351	32.3
2	793	18.9
3	348	8.3
4	156	3.7
5	58	1.4
6	28	0.7
7	15	0.4
8	6	0.1
9	3	0.1
10	1	—
12	1	—

Total number of drugs used: 5265. Mean number of drugs used by all subjects: 1.3. Mean number of drugs used by women taking at least one medication: 2.9.

No previous research, to our knowledge, has assessed the relationship of medication use with maternal characteristics other than age and race, or to exposures other than smoking. We are also unaware of prior research into either the relationship of maternal characteristics and drug use for over-the-counter and prescription drugs separately, or studies looking at the relationship between over-the-counter and prescription drug use. This study addresses these questions in a relatively large population of pregnant women.

Material and methods

All women who made their first prenatal visit to a private obstetric or midwifery practice, a health main-

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Table II. Prevalence of over-the-counter and prescription drug use, Yale–New Haven Hospital, 1980 to 1982

No.	*Over-the-counter drugs**		*Prescription drugs†*	
	Frequency	*%*	*Frequency*	*%*
0	1887	45.1	3043	72.7
1	1444	34.5	802	19.2
2 or 3	768	18.4	302	7.2
≥4	87	2.1	39	0.9
TOTAL‡	4186	100.1	4186	100.0

*Total number of over-the-counter drugs used = 3542; average number of over-the-counter drugs for all subjects = 0.9.

†Total number of prescription drugs used = 1638; average number of prescription drugs for all subjects = 0.4.

‡Total number of drugs = 5180. Eighty-five drugs could not be labeled over-the-counter or prescription and are not used in those frequency tables or analyses that look at over-the-counter and prescription drugs separately.

Table III. Cross-tabulation of over-the-counter and prescription drug use, Yale–New Haven Hospital, 1980 to 1982

Over-the-counter drug use	*Prescription drug use*			
	0	*1 or 2*	*3 or 4*	*>5*
0	1440	411	28	0
1 or 2	1423	554	46	7
3 or 4	155	68	13	1
>5	13	10	4	0

$\chi^2 = 60.66$; $p < 0.0001$.

tenance organization, or a hospital clinic, and who were scheduled for delivery at Yale–New Haven Hospital, New Haven, Connecticut, were invited to participate in the study. Subjects were recruited between May 12, 1980, and March 12, 1982. Of 6219 women identified at the start of the study, 4926 were willing to be contacted and met the eligibility criteria. Valid interviews were completed for 4186 (85%) of the eligible subjects. The interview was conducted by trained interviewers and contained questions concerning pregnancy history, demographic characteristics, and exposure to other possible risk factors. Most interviews took place in the woman's home, the majority a few weeks after the first prenatal visit. Of all subjects, 80% were interviewed before week 22 of the pregnancy. A more detailed description of subject selection and data collecting procedures has been provided elsewhere.[7]

To ascertain their exposure during the critical period of organogenesis, women were asked whether they had taken any medication in each of the 12 weeks since their pregnancy started (i.e., since the estimated day of conception) for any specific indication. A list of 23 different indications such as fluid retention, nausea, constipation, and epilepsy was read to the respondent. For every affirmative answer the name of the specific drug or medication was asked. Where possible, women were asked to show the interviewer the package with the name of the medication. Over-the-counter drugs were coded in accordance with the listing from the *Handbook of Nonprescription Drugs*.[8] The prescription drug listing was coded according to the American Hospital Formulary Service.[9] Vitamins, iron supplements, and food supplements were excluded from the drug listings.

Respondents were asked whether and how often since their pregnancy started they had consumed different types of alcohol and how many drinks, on average, they had at one time. They were asked whether and how often they had used marijuana or hashish since their pregnancy started. Information on smoking was obtained by asking whether a woman had smoked during each of the months of the pregnancy and, if she had, how many cigarettes, on average, per day.

First we displayed the frequency of overall and individual use. Next, a χ^2 analysis was carried out to assess the relationship between frequency of over-the-counter and prescription drug use. Further, to study whether maternal characteristics influence the extent of drug use, analyses were carried out to assess frequency of medication use by a number of maternal variables, both for all drugs and for prescription and over-the-counter drugs separately. Finally, we carried out logistic regression analyses with the use of the Statistical Analysis System (SAS)[10] to assess the independent relationship of maternal characteristics to prescription, over-the-counter, and total use of drugs.

Results

Of the 4186 study subjects, 67.0% were younger than 30 years of age, 78.1% were white, 78.2% were married, 58.7% had more than 12 years of education, and 46.0% were nulliparous. During the first trimester of pregnancy, 34.1% of women abstained from medication use, 32.3% used one drug, 27.3% used two or three, and

Table IV. Frequency of over-the-counter drug use by indication category, Yale–New Haven Hospital, 1980 to 1982

*Indication category**	*Frequency*	*% All OTC uses*	*% All drug uses*	*% All women*
Internal analgesics	2181	61.6	42.1	52.1
Antacids	361	10.2	7.0	8.7
Cold, allergy	313	8.8	6.0	7.5
Laxatives	150	4.2	2.9	3.6
Antitussives	130	3.7	2.6	3.1
Skin products	127	3.6	2.5	3.0
Antidiarrheals	72	2.0	1.4	1.7
Weight-control medication	54	1.5	1.0	1.3
Topical decongestants	50	1.4	1.0	1.2
Appetite suppressants	43	1.2	0.8	1.1
Antiemetics	18	0.5	0.3	0.3
Menstrual products	14	0.4	0.3	0.3
Throat lozenge	13	0.4	0.3	0.3
Sleep aid	8	0.2	0.2	0.2
Asthma medication	6	0.2	0.1	0.2
Stimulants	5	0.1	0.1	0.1

OTC, Over-the-counter.
*Indications coded by the *Handbook of Nonprescription Drugs*.[8]

6.4% used four or more drugs. A total of 5265 drug uses was recorded, an average of 1.3 per woman. Among all women using at least one medication, 2.9 drugs were used on average (Table I). Of the total of 5265 reported drug uses, 5180 (98.4%) were coded by the interviewer and could subsequently be classified as a prescription or over-the-counter drug. Table II shows the frequency of drug use in the two categories. Of all women, 27.3% used prescription drugs, 19.2% used one, 7.2% two or three, and 0.9% four or more. A total of 1638 prescription drugs were used, an average of 0.4 per woman for the entire cohort. Of all women, 54.9% used over-the-counter drugs, 34.6% used one, 18.4% two or three, and 2.1% four or more. A total of 3542 over-the-counter drugs were used, an average of 0.9 per woman for the entire cohort.

We cross-classified use of over-the-counter and prescription drugs (Table III). The association between frequency of prescription and over-the-counter drugs was highly significant ($p < 0.0001$), indicating that women who use prescription medication are also more likely to use over-the-counter medications and vice versa.

Over-the-counter drugs were grouped into their indication categories and the frequency of use in each was calculated (Table IV). Internal analgesics are the most frequently used and are used by more than half of all the women in this cohort. They also account for more than 40% of all drugs used in this study. Table V shows the frequency of prescription drug use. Autonomic drugs were most frequently used. Of all women in the cohort, 10% used autonomic drugs and they constituted 27.5% of all prescription drug use.

Table VI shows the most frequently used over-the-counter and prescription drugs. Among the over-the-counter preparations, Tylenol analgesic is used most, accounting for more than 40% of all nonprescription drugs, and used by 36.5% of all women in the cohort. The most frequently used prescription drugs are the parasympatholytic agents (25% of all prescription drugs), of which Bendectin antihistamine was the single most prescribed drug, used by 9.2% of all women in the cohort.

Table VII shows the distribution of maternal characteristics by drug exposure. Of all women 31.9% smoked at least one cigarette a day at the time of interview, 69.3% drank some alcohol, 76.8% had exposure to caffeine, and 9.7% used marijuana. Women who used drugs were significantly more likely to be older, married, white, and college-educated. They were also more likely to have consumed alcohol, caffeine, and marijuana. No relationship was found between frequency of drug use and cigarette smoking or employment status.

A similar analysis for over-the-counter drugs was performed. We found similar associations with older age, being married, being white, being college-educated, use of caffeine, alcohol, and marijuana, and more frequent use of over-the-counter drugs. Prescription drugs were also similarly analyzed. Again the association with age, marital status, ethnicity, education, caffeine use, and marijuana use were significant. No relationship was found between alcohol use and pre-

Table V. Frequency of prescription drug use by category, Yale–New Haven Hospital, 1980 to 1982

Category	*Frequency*	*% All prescription uses*	*% All drug uses*	*% All women*
Autonomic drugs	452	27.6	8.7	10.8
Parasympatholytics	410			
Sympathomimetics	29			
Sympatholytics	4			
Skeletal muscle	8			
Relaxants	8			
Antiinfective agents	274	16.7	5.3	6.5
Cephalosporins	9			
Erythromycin	37			
Penicillin	157			
Tetracyclin	15			
Others	19			
Antituberculosis	1			
Sulfonamides	18			
Trichomonacides	7			
Urinary germicides	7			
Sulfones	1			
Central nervous system drugs	273	16.7	5.3	6.5
Analgesics, antipyretics	112			
Antidepressants	7			
Tranquilizers	69			
Respiratory stimulants	14			
Sedatives	42			
Skin and mucous membrane preparations	167	10.2	3.2	4.0
Synthetic hormones	116	7.1	2.2	2.7
Adrenals	31			
Estrogens	4			
Insulins	10			
Thyroid, antithyroid	62			
Parathyroid	1			
Progestagens	16			
Gastrointestinal drugs	91	5.5	1.7	2.2
Antacids	9			
Antidiarrheals	6			
Antiflatulents	7			
Laxatives	17			
Emetics/antiemetics	43			
Miscellaneous	4			
Antihistamines	102	6.2	2.0	2.4
Electrolyte, caloric, and water balance	51	3.1	1.0	1.2
Replacement	2			
Diuretics	47			
Expectorants	44	2.7	0.8	1.1
Eye, ear, nose, throat	36	2.2	0.7	0.8
Antibiotics	4			
Antiinflammatory	7			
Local preparations	25			
Spasmolytic agents	29	1.8	0.6	0.7
Cardiovascular drugs	20	1.2	0.4	0.5
Cardiac drugs	8			
Hypotensive agents	12			
Local anesthetics	8	0.5	0.2	0.2
Unclassified	6	0.4	0.1	0.1

Indications coded by the American Hospital Formulary Service.[9]

scription drug use. No relationship was found between cigarette smoking and over-the-counter or prescription drugs. Employment status was only significant for over-the-counter drug use, with employed women being more likely to be frequent drug users.

Logistic regression analysis was used to estimate the independent effects of age, education, ethnicity, employment, marital status, marijuana use, smoking and alcohol use on total medication use. The variables that were retained in the model are shown in Table VIII. White women, women smoking more than 20 cigarettes per day, and those using alcohol or caffeine are significantly more likely to use medications.

Comment

This study indicates that two thirds of pregnant women take some medication during the first trimester of pregnancy, the period during which the fetus is most

Table VI. Most frequently used over-the-counter, grouped prescription, and individual prescription drugs, Yale–New Haven Hospital, 1980 to 1982

Drug	*Frequency*	*% All prescriptions, over-the-counter uses*	*% All drug uses*	*% All women*
Over-the-counter preparations				
Tylenol analgesic	1526	43.1	29.5	36.5
Acetylsalicylic acid enseals	297	8.4	5.7	7.1
Maalox suspension	101	2.9	1.9	2.4
Bufferin analgesic	78	2.2	1.5	1.9
Robitussin expectorant	70	2.0	1.4	1.7
Excedrin analgesic	66	1.9	1.3	1.6
Sudafed decongestant	62	1.8	1.2	1.5
Pepto-Bismol upset stomach remedy	55	1.6	1.1	1.3
Prescription drugs by subgroup				
Parasympatholytics	410	25.0	7.9	9.8
Penicillin	157	9.6	3.0	3.8
Analgesics	133	8.1	2.6	3.2
Antihistamines	64	3.9	1.2	1.5
Thyroid, antithyroid	62	3.8	1.2	1.5
Tranquilizers	62	3.8	1.2	1.5
Diuretics	48	2.9	0.9	1.1
Expectorants	46	2.8	0.8	1.1
Emetics, antiemetics	44	2.7	0.8	1.1
Antiinflammatory	41	2.6	0.8	1.0
Individual prescription drugs				
Bendectin antihistamine	385	23.5	7.4	9.2
Penicillin	157	9.6	3.0	3.8
Aspirin	73	4.5	1.4	1.7
Diazepam	69	4.2	1.3	1.6
Ampicillin	61	3.7	1.2	1.5
Acetaminophen	46	2.4	0.9	1.1
Erythromycin	35	2.1	0.7	0.8
Compazine	23	1.4	0.4	0.5
Fiorinal	20	1.2	0.4	0.5
Hydrochlorothiazide	18	1.1	0.3	0.4

Indications coded by the *Handbook on Nonprescription Drugs*[8] and the American Hospital Formulary Service.[9]

susceptible to teratogenic effects. The number of women using medications is similar to that found in prior studies[1-6] although the average number of drugs consumed seems to be slightly lower in our study. Earlier studies found that 43.6% to 92.0% of all women used at least one drug, with an average of 3.0 to 5.5 drugs being used throughout the pregnancy among women using drugs. Our findings were that 55.9% of all women used at least one drug. An average of 1.25 drugs were used during the first trimester by all women and an average of 1.9 by those using at least one medication. It is possible that we found a lower average because, unlike some other studies, we only monitored use during the first trimester or because of other differences in the study population.

The drugs most frequently reported in our study were internal analgesic products, parasympatholytic agents, penicillin, antitussives, and cold and allergy preparations. The same findings were reported in earlier studies.

Use of prescription and over-the-counter drugs was significantly related: women who used drugs from one group were also more likely to use drugs from the other. This is an interesting finding given that differences exist in the way each type of medication is obtained. It is possible that women who suffer from certain conditions that require prescription medication are also more likely to buy over-the-counter drugs for the same condition. It is also possible that a certain "medication-taking behavior" exists: women who use over-the-counter drugs might also be more likely to obtain prescription drugs. Yet another explanation might be economic: women who cannot afford medications are likely to not use either over-the-counter or prescription drugs.

Our analyses show that there do not seem to be major differences in the maternal characteristics of women who frequently use over-the-counter versus prescription drugs during pregnancy. In this analysis, the maternal characteristics associated with low frequency of drug use were being nonwhite, unmarried, and less educated. Being unemployed was significantly related to greater over-the-counter drug use but not with prescription drug use. Unemployed women may be less able to afford regular physician office visits and, consequently, be less likely to receive a prescription. Of the risk-taking behaviors known to be associated with adverse pregnancy outcome (especially low birth weight),

Table VII. Overall drug exposure by maternal characteristics, Yale–New Haven Hospital, 1980 to 1982

Maternal characteristic and significance test	*No. 4185*	*Overall drug exposure*			
		None (n = 1425)	*1 (n = 1351)*	*2 or 3 (n = 1141)*	*4> (n = 268)*
Age (yr)*					
<20	414	14.25	10.36	5.78	1.87
20-24	933	23.51	22.95	20.68	19.40
25-29	1539	33.68	37.23	39.79	38.06
30-34	1074	23.30	25.61	27.34	31.34
≥35	225	5.26	3.85	6.40	9.33
Marital status†					
Currently married	3269	72.61	77.48	83.25	89.55
Divorced, separated, widowed	155	3.23	4.00	4.12	2.99
Single	758	24.16	18.52	12.63	7.46
Ethnicity‡					
White	3261	69.65	79.19	83.42	94.38
Black	761	25.63	17.04	13.60	4.49
Other	155	4.72	3.78	2.98	1.12
Education (yr)§					
<12	565	19.09	13.55	8.33	5.60
12	1162	28.42	28.42	27.61	21.64
13-16	1787	37.26	42.56	47.85	50.37
≥17	671	15.23	15.47	16.21	22.39
Current employment‖					
Employed	2143	55.52	55.67	58.45	66.55
Not employed	1634	44.48	44.33	41.55	39.45
Cigarettes smoked¶					
Nonsmoking	2871	69.52	66.79	69.77	69.92
1-10/day	790	19.45	20.90	17.31	12.78
10-20/day	405	9.41	9.86	9.31	12.03
>20/day	111	1.62	2.45	3.60	5.26
Alcohol (oz/day)#					
None	1287	38.60	28.13	26.03	22.39
≤0.25	2558	54.46	63.88	65.73	63.06
>0.25	340	6.95	7.99	8.24	14.55
Caffeine (mg/day)**					
None	972	27.58	20.87	21.03	21.27
1-150	2057	46.60	50.19	51.01	49.63
151-300	807	17.82	20.80	19.46	18.66
≥301	349	8.00	8.14	8.50	10.45
Marijuana use††					
No	3775	91.36	89.70	91.23	83.58
Yes	406	8.64	10.30	8.77	16.42

*$\chi^2 = 98.4$; $p < 0.0001$.
†$\chi^2 = 80.5$; $p < 0.0001$.
‡$\chi^2 = 121.6$; $p < 0.0001$.
§$\chi^2 = 100.3$; $p < 0.0001$.
‖$\chi^2 = 4.1$; $p = 0.25$.
¶$\chi^2 = 3.2$; degrees of freedom = 3; $p = 0.366$.
#$\chi^2 = 78.187$; degrees of freedom = 6; $p < 0.0001$.
**$\chi^2 = 25.60$; degrees of freedom = 9; $p = 0.002$.
††$\chi^2 = 17.27$; degrees of freedom = 3; $p = 0.001$.

marijuana use was significantly related to greater frequency of drug use in all analyses. Increased alcohol drinking was associated with over-the-counter and overall drug use but not with prescription drug use. Cigarette smoking was related to prescription drug use as well as overall drug use. It was not related to over-the-counter drug use. White women were more likely to use both types of medications. White women may be better able to afford medications than nonwhite women, but a related explanation could be that they are more likely to receive prenatal care and to subsequently have medications prescribed to them. If this explanation indeed holds true, improvement in prenatal care may diminish the difference in frequency of drug use between white and nonwhite women. However, when a separate analysis was carried out on race and frequency of use of Bendectin antihistamine, women in all racial groups used Bendectin antihistamine with the same frequency. Bendectin antihistamine was the most frequently used prescription drug, prescribed for nausea in pregnancy. If receiving prenatal care is indeed related to frequency of prescription drug use, use of Bendectin antihistamine would have been expected to be higher among white women.

The analysis shows that women who are white, use marijuana, are heavy smokers, drink alcohol, and use caffeine were more likely to use medications. These are independent risk factors and the observed link between more frequent medication use and other risk-taking behaviors occurs in white and nonwhite women. It is possible that a woman's use of cigarettes, marijuana, caffeine, or alcohol might directly influence her medication-taking behaviors. Women might become more aware of the possible dangers of medication use during pregnancy, just as they are of the hazards of smoking or drinking. Women who try to avoid any dangerous exposures when pregnant might also avoid medication use. Alternatively, physicians may be more likely to prescribe to certain women because of their social characteristics or medical condition. Cigarette smoking, for instance, has been related to a greater risk of upper respiratory problems. These conditions may require increased medication use.

Table VIII. Final model for logistic regression analysis on total drug use, Yale–New Haven Hospital, 1980 to 1982

Maternal characteristic	*Odds ratio*	*95% Confidence interval**
White race	0.58	0.46-0.71
Smokes 1-10 cigs/day	1.03	0.83-1.28
Smokes 11-20 cigs/day	0.92	0.71-1.19
Smokes >20 cigs/day	1.92	1.11-3.23
Alcohol use	1.39	1.19-1.62
Caffeine use 1-150 mg/day	1.23	1.04-1.46
Caffeine use >150 mg/day	1.23	1.01-1.85

Cigs, Cigarettes. Reference categories are being nonwhite, nonsmoking, and not using alcohol or caffeine.

Calculated by: $e^{(\beta \pm 1.96 \times SE\ \beta)}$

Because so little is known about the teratogenic potential of most drugs (prescription as well as over-the-counter drugs) only those drugs should be used for which a large body of information is available, and then only when necessary. Clearly, some drugs, such as antiepileptic agents, may need to be taken even without full knowledge of their teratogenic potential. However, this study indicates that the great majority of drug use in pregnancy is not for serious conditions. Bendectin antihistamine was the single most widely used prescription drug in the study. As a result of numerous studies,[11] Bendectin antihistamine is now generally recognized not to be teratogenic. However, it is ironic (given that Bendectin antihistamine has been withdrawn from the market) and sobering to realize that Bendectin antihistamine was in widespread use long before evidence of its safety was well documented. Physicians should be careful when prescribing to pregnant women and should always inquire about the possibility of pregnancy before prescribing to any woman of childbearing age. Pregnant women should learn to be more critical toward the use of drugs and should first try alternative treatments for common pregnancy discomforts. For example, Bendectin antihistamine was used by 30% of women in some reports, which would appear to be well above the proportion of women who suffer serious nausea and vomiting in early pregnancy.[11] This overuse implies underuse of nonpharmacologic treatment in mild cases.

Aspirin was the second most frequently used over-the-counter drug in this cohort. Aspirin affects prostaglandin synthesis and given before delivery, it can, even in low doses, affect the clotting ability in the newborn.[13] The relationship between aspirin and congenital birth defects remains controversial, in spite of the fact that several studies have been undertaken.[2, 14, 15]

Acetaminophen was the most widely used of all drugs in this study. There is no evidence of possible teratogenic properties[2] and, unlike aspirin, acetaminophen does not increase the risk of fetal hemorrhage when given to the mother at term.[16] All this considered, acetaminophen is recommended when an analgesic or antipyretic is needed in pregnancy. This means that in the case of aspirin use in this cohort we know that an equally effective, but possibly less harmful alternative, would have been available. In view of our still inadequate knowledge about the safety of most drugs, pregnant women should be counseled about the potential hazards of medication use during pregnancy. Ideally, a woman should stop taking drugs as much as possible as soon as she plans to become pregnant or when she suspects she might be pregnant.

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Extensive pulmonary embolism presenting as severe adult respiratory distress syndrome after surgical resection of a cornual pregnancy

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A rare case of severe adult respiratory distress syndrome associated with a Po_2 of 9 mm Hg after surgical resection of a cornual pregnancy at 10 weeks' gestation was treated successfully with positive end-expiratory pressure. The underlying cause, extensive pulmonary embolism, was subsequently revealed. The patient survived without residual brain damage. The possible causes of adult respiratory distress syndrome in pregnancy are discussed. (AM J OBSTET GYNECOL 1991;165:41-2.)

Key words: Adult respiratory distress syndrome, pulmonary embolism

Adult respiratory distress syndrome describes sudden respiratory failure as a result of noncardiogenic pulmonary edema.[1] Adult respiratory distress syndrome represents a final common pathway of lung injury because of a wide spectrum of unrelated causes resulting in increased alveolar-capillary permeability. Adult respiratory distress syndrome is characterized by progressive respiratory distress, severe hypoxemia relatively refractory to high inspired oxygen tension, low lung compliance, and diffuse infiltrates on chest roentgenogram, and carries a high mortality rate of between 50% to 80%.[2] This syndrome has been well documented in association with massive trauma and shock.[1] Over the past decade, adult respiratory distress syndrome has also been recognized in pregnancy.[1, 2] We present a rare case of extensive pulmonary embolism presenting as severe adult respiratory distress syndrome in a woman after surgical resection of a cornual pregnancy at 10 weeks' gestation in the absence of shock.

Case report

A 32-year-old, gravida 4, para 2 woman was first seen with abdominal pain in the right lower quadrant and irregular vaginal bleeding. On physical examination she was hemodynamically stable; blood pressure was 102/58 mm Hg and pulse was 82 beats/min. The abdomen was tender with no signs of peritoneal irritation. On pelvic examination the uterus were boggy and enlarged to 6 weeks' size and the right adnexa were tender with no palpable mass. Laboratory study results were within normal limits with a hematocrit value of 32%. A urine β-human chorionic gonadotropin test result was positive. Sonographic examination showed a gestational sac that measured 4.5 × 4.4 cm and contained a fetal pole with no fetal heart motion located in the right cornual area of the uterus. Laparoscopy confirmed the diagnosis of a cornual pregnancy and at laparotomy right cornual resection with right salpingectomy was performed. Twelve hours after surgery a hematocrit value of 20% was noted. Sonography revealed free fluid in the abdomen. The patient was transfused with 2 units of blood and at exploratory laparotomy the uterine incision, which was found to be bleeding, was sutured. Six hundred milliliters of clotted blood was removed from the abdomen. Two additional units of blood were transfused. The patient remained hemodynamically stable throughout surgery and platelet counts and coagulation study results were normal. The first 30 hours after the second surgical intervention were uneventful. Shortly thereafter the patient began to show signs of anxiety. At that time vital signs were stable. However, 15 minutes later she was noted to be unresponsive with profuse sputum production. Blood pressure was 108/64 mm Hg and pulse was 88 beats/min. Electrocardiogram revealed normal sinus rhythm with no abnormalities. Arterial blood gas showed pH 7.19, Pco_2 55 mm Hg, Po_2 9 mm Hg, oxygen saturation 6%. An immediate repeat analysis was similar (7.15, 60, 18, 22%, respectively). The patient was intubated and 100% oxygen administered. Chest roentgenogram revealed diffuse pulmonary infiltrates

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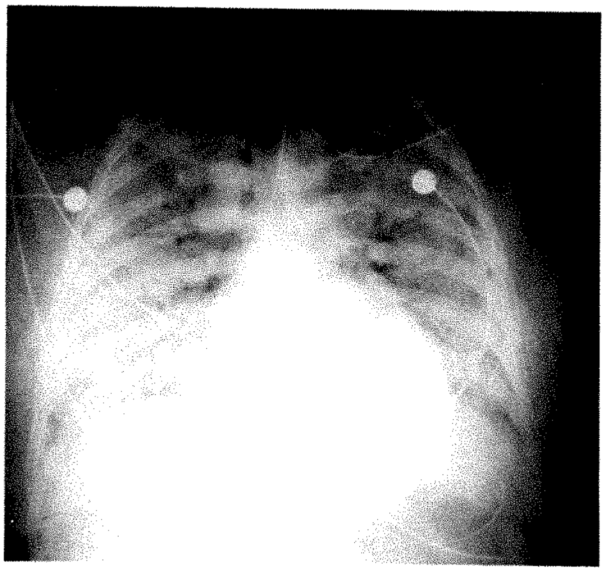

Fig. 1. Anterior-posterior chest roentgenogram performed immediately after intubation after acute presentation. Note diffuse pulmonary infiltrates throughout both lungs.

throughout both lungs (Fig. 1). With the diagnosis of adult respiratory distress syndrome, she was transferred to the Surgical Intensive Care Unit, where she was paralyzed and ventilated (settings: FIO_2 100%, positive end-expiratory pressure 15 mm H_2O, intermittent mandatory ventilation 16/min, tidal volume 800 ml). A Swan-Ganz catheter was placed with the following pressures: right atrium 16 mm Hg, pulmonary artery 45/30 mm Hg, wedge 15 mm Hg, cardiac output 7.8 L/min, cardiac index 4.2, systemic vascular resistance 749 dyne · sec · cm^{-5}, ruling out a cardiac cause for this clinical presentation.

Subsequently, a ventilation perfusion scan showed multiple pulmonary emboli. Intravenous heparin therapy was initiated. Bilateral lower extremity color duplex venous scans were normal. Multiple cultures obtained from blood, sputum, and urine were negative. Thirteen days after the initial respiratory event, after gradually being weaned off the ventilator, the patient was extubated and Coumadin anticoagulant therapy was initiated. Three days later, the patient was discharged in good health with no signs of residual brain damage.

Comment

Among the multiple disorders associated with adult respiratory distress syndrome, the most common are sepsis, gastric aspiration, massive trauma, fat embolism, pneumonia, and disseminated intravascular coagulation.[1] Although documented in pregnancy in conjunction with preeclampsia, eclampsia, amniotic fluid embolism, fetal death in utero, abruptio placentae, chorioamnionitis, sickle cell crisis, trophoblastic disease, and pyelonephritis, the most common cause of adult RDS in pregnancy is that associated with tocolysis.[1] This has been noted to occur after administration of terbutaline sulfate, ritodrine, isoxsuprine hydrochloride, and fenestrel, especially with aggressive intravenous hydration.[1] In our case, severe adult respiratory distress syndrome was apparently caused by extensive pulmonary embolism in the absence of hemorrhagic shock, sepsis, diffuse intravascular coagulation or overhydration. Survival without brain damage despite a PO_2 of 9 mm Hg is remarkable. When presented with dyspnea, tachypnea, and progressive hypoxemia even in the absence of classic underlying causes, the obstetrician-gynecologist should be alert to the possibility of adult respiratory distress syndrome. Rapid diagnosis and immediate treatment with positive end-expiratory pressure ventilation, preferably with multidisciplinary assistance in conjunction with treatment of the underlying disease, will clearly benefit these critically ill patients.

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Ten years after cryosurgical treatment of cervical intraepithelial neoplasia

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From 1974 to 1977 we treated 96 patients for grade 2 or 3 cervical intraepithelial neoplasia with cryotherapy. They were monitored for 10 to 14 years with an attendance rate of 98%. Treatment resulted in cure in 92%. Nine patients had persisting lesions, and three patients had a recurrent lesion. The last recurrence was diagnosed 6.7 years after cryotherapy. (Am J Obstet Gynecol 1991;165:43-5.)

Key words: Cryosurgery, cervical intraepithelial neoplasia, long-term follow-up

The prevalence of cervical intraepithelial neoplasia has more than doubled during the last decade and has now reached 18.8 per thousand among teenagers.[1] Cryotherapy was introduced late in the 1960s as a less destructive treatment than surgical conization.[2] Invasive cervical cancer takes about 10 years to develop from cervical intraepithelial neoplasia[3]; therefore it is possible to assess the efficacy of cryotherapy only after this period. We have monitored 96 patients for at least 10 years after cryotherapy grade 2 or 3 cervical intraepithelial neoplasia.

Material and methods

Ninety-six patients were diagnosed as having grade 2 or 3 cervical intraepithelial neoplasia from 1974 to 1977 and were treated by cryotherapy. They all had a visible transformation zone, and endocervical curettage displayed no cervical intraepithelial neoplasia. The actual age distribution and grade of cervical intraepithelial neoplasia are given in Table I.

Nitrous oxide was used as the cryogenic for cryotherapy. The diameter of the cone-shaped cryoprobe was 19 mm, and the height of the conus was 16 mm. The probe was applied for two 3-minute periods. For patients 1 through 25 the pause was 1 to 3 months. For the remaining patients the pause was 5 minutes.

After cryocoagulation, cytologic examination was carried out four times during the first year, twice during the second year, and thereafter once a year. After the fifth year the cytologic controls were done by the patient's own general practitioner.

Colposcopic examination was routinely performed after 3 months; 1, 3, and 5 years; and when indicated by cytologic testing.

The result of each woman's most recent smear test was obtained from her general practitioner. If the latest smear test was performed <10 years after cryotherapy, the patient was referred for cytologic control.

If cervical intraepithelial neoplasia was diagnosed

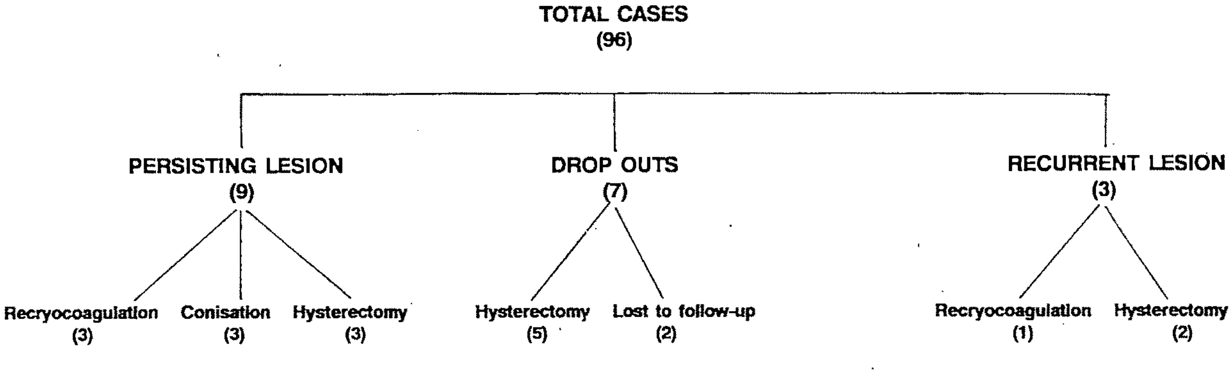

Fig. 1. Follow-up and outcome 10 years after cryotherapy for grade 2 and 3 cervical intraepithelial neoplasia.

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Table I. Comparison of histologic diagnosis with patient age and cryotherapy efficacy

Grade of cervical intraepithelial neoplasia	*Patients (No.)*	*Age (yr, mean and range)*	*Persisting lesion* (No.)*	*Recurrent lesion* (No.)*
2	25	30.6 (19.2-51.0)	2 (1)	0
3	71	31.4 (21.2-51.6)	7 (2)	3 (1)
TOTAL	96	31.2 (19.2-51.6)	9 (3)	3 (1)

*Number in parentheses represent number of patients treated by recryocoagulation.

Table II. Treatment failures after first cryotherapy. A life table, with 95% confidence limits of life table product

Years after treatment	*Failure (No.)**	*Patients at risk (No.)*	*Cumulative success rate (%)*	*Confidence limits (%)*
0-1	9 (3)	88	90.6	84.8-96.4
1-2	0	87	90.6	84.8-96.5
2-3	1	86	89.6	83.5-95.7
3-4	1 (1)	85	88.5	82.1-94.9
4-5	0	84	88.5	82.1-94.9
5-6	0	82	88.5	82.0-95.0
6-7	1	79	87.4	80.6-94.2
7-8	0	78	87.4	80.5-94.3
8-9	0	77	87.4	80.5-94.3
9-10	0	76	87.4	80.4-94.4
10-11	0	66	87.4	79.9-94.9

*Number in parentheses represents number of patients treated by recryocoagulation.

during the first year after cryotherapy, the lesion was defined as persisting. If this diagnosis was obtained later in the study period, the lesion was defined as recurrent. Treatment by recryocoagulation was accepted in all cases.

Statistical calculations were based on Fischer's test, with a significance level of $p < 0.05$.

Results

After the first cryotherapy nine patients had a persisting lesion. After recryocoagulation, six still had a persisting lesion; three of them were treated with conization and three with hysterectomy. There was no significant difference ($p = 0.703$) between grade 2 and grade 3 cervical intraepithelial neoplasia (Table I).

Follow-up for 10 years failed in seven patients. Two did not volunteer in the control program; however, the most recent smears obtained from these patients (67 and 82 months, respectively) after the cryotherapy were normal. Five patients had hysterectomy for reasons other than cervical intraepithelial neoplasia (the average time of control for these patients was 62 months), and they were all shown to have normal histologic features of the cervix.

One patient had persisting lesions despite undergoing cryotherapy three times. She refused to undergo conization, and a lethal invasive cervical cancer developed 3.6 years after the first cryocoagulation.

Three other patients had a recurrent lesion after 3, 3.7, and 6.7 years, respectively (Table II). One of these patients was treated by recryocoagulation and two underwent hysterectomy (Fig. 1).

Comment

A 92% effectiveness after cryotherapy of grade 2 or 3 cervical intraepithelial neoplasia is in agreement with other studies,[4-7] despite the fact that these studies have a lower attendance for follow-up than the 98% (2/96) in this investigation. Most of the treatment failures were persisting dysplasia (9/12, 75%). Only three showed recurrent dysplasia. Therefore follow-up with intensive control during the first year after cryotreatment is strongly recommended.

Treatment of grade 2 and 3 cervical intraepithelial neoplasia was equally successful. This is in agreement with Townsend and Richart,[8] who found that the effectiveness of cryotherapy is dependent on the area of the lesion and not the degree.

Overlooked invasive growth at the time of treatment is the most frequent reason for the apparent development of cervical cancer after cryotherapy.[9, 10] We underscore the importance of colposcopic examination, endocervical curettage, and colposcopically directed cervical biopsy of the most advanced lesions.

The observed failure rate of 13% (12/96) in this study and up to 20% in other studies[10] and the possibility of developing cervical cancer, documented both in this study and in others,[11-13] emphasize the necessity of being absolutely sure that the patients to whom we offered cryotherapy agreed to the follow-up controls.

In conclusion, cryotherapy is economical and gentle and can be done on an outpatient basis without anesthesia. We find cryotherapy to be as effective for the treatment of cervical intraepithelial neoplasia as electrocauterization and the more expensive and laborious laser therapy.[8, 14-16]

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Recurrence of ectopic pregnancy: The role of salpingitis

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We evaluated the role of salpingitis on the recurrence of ectopic pregnancy from a historical cohort of 2501 women who had undergone laparoscopic examination for acute salpingitis. We used pregnancy ($N = 2899$) as the unit of analysis and a modified conditional logistic regression to estimate a pairwise odds ratio as a measure of the recurrence of ectopic pregnancy. Among the second or higher order of pregnancy, the recurrence was 21.7%. For pregnancies with a prior uterine pregnancy, the ectopic pregnancy rate increased with prior salpingitis scores constructed from a combination of prior salpingitis episodes and severity (0 score, 2.7%; 1 to 2 scores, 4.8%; and ≥ 3 scores, 12.1%). For those with a prior ectopic pregnancy, the rate did not increase with prior salpingitis scores (score 0, 20.0%; score 1 or 2, 19.2%; and score ≥ 3, 26.9%). The adjusted pairwise odds ratio was 2.2 and was practically unchanged (2.1) after additional adjustment with prior salpingitis scores. These findings confirm salpingitis as a risk factor for first ectopic pregnancy, but once a woman had an ectopic pregnancy, previous salpingitis might not add any incremental risk. (Am J Obstet Gynecol 1991;165:46-50.)

Key words: Salpingitis, ectopic pregnancy

Ectopic pregnancy, a sequel of pelvic inflammatory disease, has increased worldwide.[1] Ectopic implantation not only affects the current pregnancy, but also is associated with recurrence in subsequent pregnancies. The estimated recurrence rate of ectopic pregnancy ranges from 10% to 20%.[2-4] A number of case control studies have reported an increased risk of ectopic pregnancy for those with a prior history of this condition, with odds ratios ranging up to ninefold.[5, 6] While previous cohort studies have documented a strong association between earlier salpingitis episodes and the initial ectopic pregnancy,[7, 8] no studies have investigated the combined role of previous salpingitis and ectopic pregnancy on the recurrence of ectopic pregnancy. In this study, we evaluated the role of salpingitis before and after the initial ectopic pregnancy on the recurrence of ectopic pregnancy from a large historic cohort study, with pregnancy used as the unit of analysis.

Material and methods

The study population consisted of a cohort of 2503 women, <36 years old, who underwent a laparoscopic examination at the Department of Obstetrics and Gynecology, University Hospital, Lund, Sweden, from Jan. 1, 1960, through Dec. 31, 1984. Briefly, the University Hospital is the only hospital in the city of Lund for inpatient treatment of gynecologic disorders. It served a female population (age 10 to 35 years) ranging from approximately 40,000 in 1960 to 50,000 in 1984. All women in this study were inpatients with signs and symptoms of acute pelvic inflammatory disease who underwent index (initial) laparoscopy within 24 hours of admission. For each woman a thorough review of her fertility status after initial laparoscopy was conducted up to Dec. 31, 1984. The findings of laparoscopy were graded as follows: normal, no abnormality in the pelvic cavity; mild salpingitis, fallopian tubes reddened and swollen but freely movable with normal morphologic characteristics of fimbriated ends; moderately severe salpingitis, more advanced inflammatory changes with tubes not freely movable and abnormal or not clearly visible fimbriated ends; severe salpingitis, pelvic peritonitis or abscess formation with visibility significantly impaired by inflammatory masses (ostia were not visible).

Of the 2503 women, two were excluded because of insufficient information on date of initial laparoscopy. Of these 2501 women, 206 did not undergo complete follow-up, 19 had a history of ectopic pregnancy before initial laparoscopy, and 736 never became pregnant after initial laparoscopy (Fig. 1). The interval of follow-up for women in this cohort ranged from 1 to 315 months. Of the 1540 women who became pregnant after initial laparoscopy, 754 had been pregnant once, 403 twice, 248 three times, 93 four times, and 42 more than four times. Two pregnancies were excluded from the analysis because of insufficient information in

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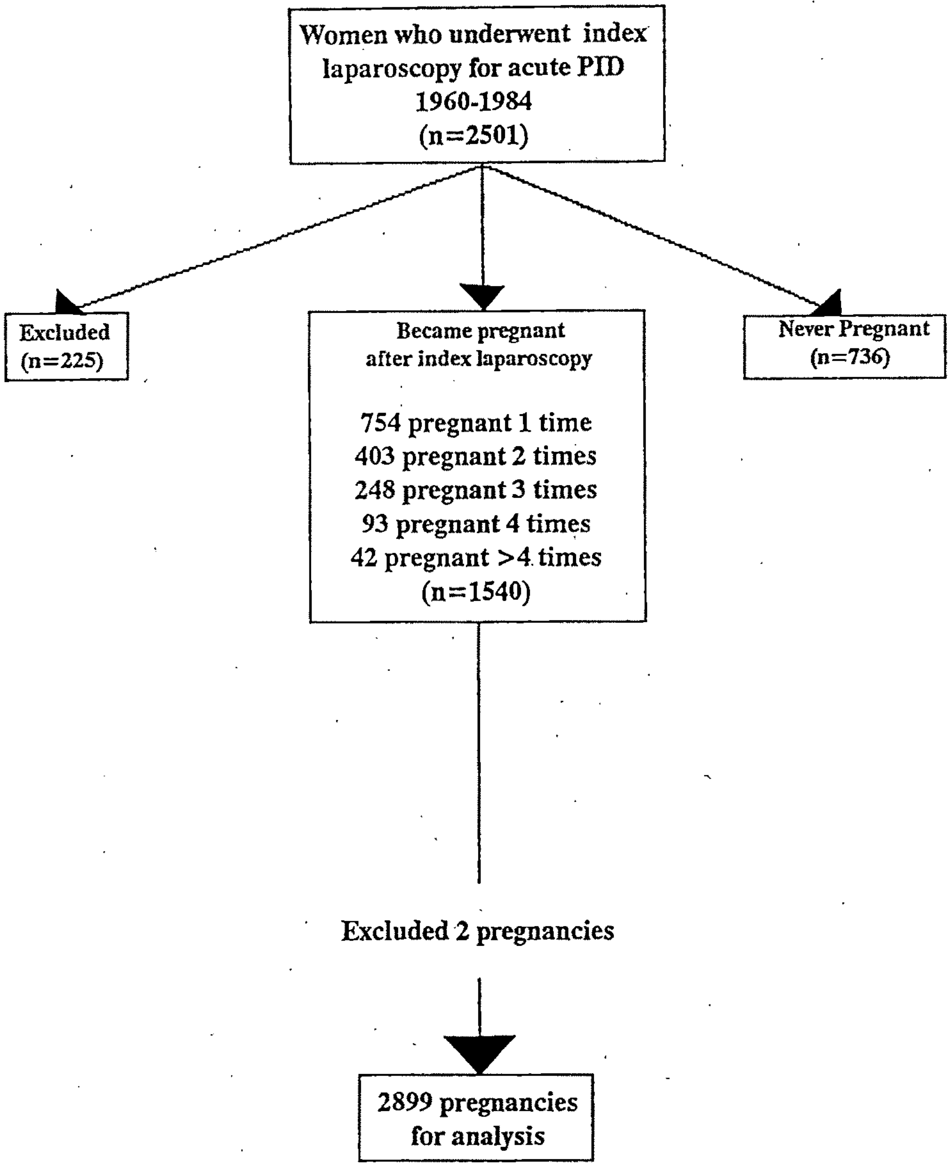

Fig. 1. Recruitment process in the Lund cohort study (see text for reasons of exclusion).

the records. A total of 2899 pregnancies were included in this analysis—1538 first-order, 786 second-order, 383 third-order, and 192 fourth-order or higher pregnancies.

Our main objective was to evaluate the role of salpingitis before and after the initial ectopic pregnancy on the recurrence of ectopic pregnancy. Because the unit of analysis is pregnancy and the pregnancy outcomes in a woman are not independent (the basic assumption of classic logistic regression), we used a modified conditional logistic regression model[9] to adjust for other known risk factors for ectopic pregnancy. This model computes both the adjusted odds ratios for risk factors and a pairwise odds ratio as a measure of the recurrence of ectopic pregnancy.

Each pregnancy could have been preceded by none, one, or several episodes of salpingitis, which had varying degrees of severity. We constructed a salpingitis score that took into account the number of salpingitis episodes and their severity (no salpingitis, score of 0; mild salpingitis, 1; moderate, 2; severe, 3). We then summed the scores for all episodes of salpingitis. For example, a pregnancy that had three prior episodes of salpingitis (mild, severe, and severe) would have a score of 7 (1 + 3 + 3). The salpingitis scores for the pregnancies ranged from 0 to 9.

For each pregnancy we evaluated and adjusted the analysis for the following risk factors: age at pregnancy (<25, 25 to 30, or >30 years), marital status (single, married, or others), number of prior pregnancies (0,

Table I. Distribution of ectopic pregnancy for selected risk factors

Risk factors	*Ectopic pregnancy*	
	No.	*%*
Marital status		
Married	84	6.7
Single	97	6.8
Others	8	6.4
Age at pregnancy (yr)		
<25	41	3.6*
25-30	101	8.5
>30	51	8.7
Prior pregnancies		
0	104	6.8
1	52	6.6
2	27	7.1
>2	10	5.2
Prior adnexal operations		
0	158	5.8*
1	26	18.4
>1	9	25.7
Prior salpingitis episodes		
0	18	2.2*
1	148	8.0
2	21	11.0
>2	6	15.4
Prior salpingitis scores		
0	18	2.2*
1-2	91	6.0
3	59	13.5
>3	25	18.1
TOTAL	193	6.7

*$p < 0.01$ for linear trend.

1, 2, or >2), prior salpingitis scores (score 0, 1 to 2, 3, or >3), and number of prior adnexal operations (0, 1, or >1). Such operations were mostly salpingostomy, salpingectomy, or salpingo-oophorectomy because of prior ectopic pregnancy. The microbiologic organisms in the salpingitis episodes could not be evaluated because routine testing for chlamydial infection was not started until 1977. Thus small numbers limited the power to differentiate gonococcal from chlamydial or other infectious causes of salpingitis.

Results

Of the 2899 pregnancies, 193 (6.7%) were ectopic pregnancies (Table I). The proportion of ectopic pregnancies increased significantly with age at pregnancy, prior salpingitis episodes, and salpingitis score (Table I). A stronger trend was found for prior adnexal operations. In addition, the proportion of ectopic pregnancies among women with no or one episode of salpingitis increased significantly with salpingitis severity (no previous salpingitis, 2.2%; mild previous salpingitis, 5.0%; moderate salpingitis, 7.7%; and severe salpingitis, 14.1%).

Of the 1538 first-order pregnancies, 104 (6.8%) were ectopic (Table II). Among second-order or higher pregnancies, 5.6% were ectopic for those with a prior uterine pregnancy, and 21.7% for those with a prior ectopic pregnancy. The risk of ectopic pregnancy increased significantly with prior salpingitis scores for first-order pregnancies and for second-order or higher pregnancies with a prior uterine pregnancy. However, the risk of subsequent ectopic pregnancy did not increase significantly with prior salpingitis scores for those with a prior ectopic pregnancy.

Except for those pregnancies occurring after a prior ectopic pregnancy, we found a statistically significant ($p < 0.01$) increase in odds ratios of ectopic pregnancy with increasing salpingitis scores (Table II). When those with score 0 are taken as a reference group, the adjusted odds ratios for scores 1 to 2 and ≥3 were 5.0 and 12.9 for first pregnancy, 1.7 and 3.9 for pregnancies with a prior uterine pregnancy, and 0.7 and 1.2 for pregnancies with a prior ectopic pregnancy, respectively. We were unable to evaluate the effect of additional salpingitis after the initial ectopic pregnancy for recurrent ectopic pregnancy because the number of such cases was too small (one case).

The pairwise odds ratio for recurrent risk of ectopic pregnancy adjusted for marital status, age at pregnancy, number of prior pregnancies, and number of adnexal operations was 2.2 (95% confidence interval, 1.6 to 3.1); it was practically unchanged at 2.1 (95% confidence interval, 1.4 to 3.0) after additional adjustment with prior salpingitis scores (data not shown). The pairwise odds ratio 2.1 means that on average the odds of a subsequent ectopic pregnancy increased 2.1 times if the prior pregnancy was ectopic (after adjustment for all the mentioned variables).

Comment

This study indicates that once a woman has had an ectopic pregnancy, her odds of having another ectopic pregnancy are doubled. Thus the risk of ectopic pregnancy as a sequela of salpingitis persists beyond the first episode. Although prior salpingitis is an important risk factor for first ectopic pregnancy (as indicated by increased adjusted odds ratios up to 12.9 for first ectopic pregnancy), it may not add incremental risks for recurrence (as indicated by low adjusted odds ratios up to 1.2 for recurrence of ectopic pregnancy). These findings emphasize the chronicity and serious nature of salpingitis once it causes an ectopic pregnancy.

The surgical treatment for the initial ectopic pregnancy may mask the effect of prior salpingitis for the subsequent ectopic pregnancy. To take into account such an effect of the surgical treatment, we conducted

Table II. Percent of ectopic pregnancy and odds ratio by pregnancy order, status of prior pregnancy, and salpingitis score

Pregnancy order	*Status of prior pregnancy*	*Prior salpingitis score*	*Ectopic pregnancy*		*Crude*		*Adjusted*	
			No.	*%*	*Odds ratio*	*95% Confidence interval*	*Odds ratio*	*95% Confidence interval*
1	—	0	6	1.4	1.0	—	1.0	—*
		1-2	50	6.3	4.9	2.1-11.4	5.0	2.1-11.7
		>3	48	15.5	13.2	5.6-31.4	12.9	5.4-30.8
		TOTAL	104	6.8	—	—	—	—
≥2	Uterine	0	10	2.7	1.0	—	1.0	—†
		1-2	32	4.8	1.8	0.9-3.7	1.7	0.8-3.6
		>3	29	12.1	4.9	2.3-10.2	3.9	1.8-8.4
		TOTAL	71	5.6	—	—	—	—
	Ectopic	0	2	20.0	1.0	—	1.0	—†
		1-2	9	19.2	0.9	0.2-5.2	0.7	0.1-4.0
		≥3	7	26.9	1.5	0.2-8.7	1.2	0.2-7.8
		TOTAL	18	21.7	—	—	—	—

*Adjusted for marital status, age at pregnancy, and number of prior adnexal operations.

†Adjusted for marital status, age at pregnancy, number of prior adnexal operations, and number of prior pregnancies.

the following analyses. First, we adjusted the analysis with the surgical treatment (as had been shown in Table II) and conducted a separate analysis excluding those with one tube removed during the surgical treatment. Both analyses produced similar results. Second, we conducted a separate analysis excluding those with recurrent ectopic pregnancy affecting the same tube as in the previous ectopic pregnancy. We suspect that the recurrent ectopic pregnancies of the same tube may be due mainly to the surgical treatment. We found among the second-order or higher pregnancies that the proportion of recurrent ectopic pregnancy slightly increased (but not significantly so, at $p = 0.05$) to correspond with prior salpingitis score (11.1% [1/9] for a score of 0, 10.0% [4/36] for a score of 1 to 2, and 15.0% [3/17] for a score of ≥3). Because of the small number of recurrent ectopic pregnancies in this population (18 cases) and the uncertainty of the carryover effect of salpingitis on the tube operated on, we should interpret these findings cautiously. Unfortunately, we were unable to evaluate the effect of additional salpingitis after the initial ectopic pregnancy because of the small number of such cases.

Biologically, the tubal damage resulting from salpingitis appears irreversible. If serious enough to cause an initial ectopic pregnancy, the same intraluminal adhesions or dysfunction remains to threaten any subsequent pregnancy. Different mechanisms may account for the tubal damage caused by either gonococcal or chlamydial infections. With gonorrhea, an endogenous tumor necrosis factor[10] or interferon gamma may be involved.[11] With chlamydia, immune-mediated tubal destruction may be stimulated by repeat infections.[12] Regardless of the precise pathophysiologic basis, the postinfection intraluminal scarring has long-lasting effects.

To our knowledge, this study is the first to document the role of salpingitis in recurrence of ectopic pregnancy, with an appropriate multivariate analysis. Previous studies were mostly based on a case-control design with the woman as the unit of observation.[1] One follow-up study of 110 patients attempting pregnancy after ectopic pregnancy showed similar results—no association between recurrent ectopic pregnancy and previous history of pelvic inflammatory disease.[4] This study, a large historic cohort study, used pregnancy, the ideal denominator for ectopic pregnancy, as the unit of observation. Unlike others, our study estimated the pairwise odds ratio, a desirable measure of association for recurrent effect of ectopic pregnancy, and adjusted this odds ratio for a set of known risk factors.

We initially adjusted the analysis for use of intrauterine contraceptive device (yes/no) and found similar results. However, we felt the information was not adequate because this information was obtained only at initial laparoscopy, not during the follow-up period. Women might use or discontinue the intrauterine contraceptive device shortly after the initial laparoscopy. Because of this uncertainty and because of similar results after adjustment for use of intrauterine contraceptive device we presented the final analysis without the adjustment for intrauterine contraceptive device use. We did not adjust the analysis for prior infertility because this information was not asked before each pregnancy (unit of analysis). However, we adjusted the analysis for two factors as surrogates for prior infertility—prior salpingitis score (an indicator of tubal patency and a strong predictor of infertility) and previous pregnancies.

Our findings on the association between previous salpingitis episodes and ectopic pregnancy confirm the previous study that used the same but smaller data set.[7,8] A population-based case-control study reported an odds ratio of 3.3 between ectopic pregnancy and history of pelvic inflammatory disease.[13] Other studies also documented similar results.[5,6] The recurrence of ectopic pregnancy reported in this study is similar to that found in other studies.[4-6]

Our data underscore the importance of public health programs in preventing the first ectopic pregnancy from occurring. As the incidence of ectopic pregnancy is increasing, the proportion of recurrent ectopic pregnancies will undoubtedly also increase. Programs to prevent sexually transmitted lower genital tract infections, specifically those causing pelvic inflammatory disease, must play a major role in stopping the chain reaction among salpingitis, first ectopic pregnancy, and recurrent ectopic pregnancy.

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The prevalence of autoantibodies during third-trimester pregnancy complicated by hypertension or idiopathic fetal growth retardation

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Lupus anticoagulant, anticardiolipin, antinuclear, anti–deoxyribonucleic acid, antithyroglobulin, and antithyroid microsomal antibodies were assayed during third-trimester pregnancy (100 normal, 100 with complications). In spite of a normal activated partial thromboplastin time in all instances, lupus anticoagulant was further investigated by three additional procedures: tissue thromboplastin inhibition time, platelet neutralization procedure, and cephalin neutralization test. The prevalence of autoantibodies in pregnancies with hypertension reaches 16% (four with lupus anticoagulant, two with anticardiolipin, and two with antithyroid microsomal antibodies), which is significantly greater than that for idiopathic fetal growth retardation (2%) (one with lupus anticoagulant antibodies) and normal pregnancies (3%) (two with antithyroglobulin and one with autithyroid microsomal antibodies) ($p < 0.01$). Autoantibodies were equally distributed between patients with gestational hypertension and those with preeclampsia. When compared with the 42 patients with hypertension and no autoantibodies, the eight patients with autoantibody had a more frequent history of fetal growth retardation ($p < 0.05$), but there was no difference in the severity of hypertension, the frequency of obstetric complications, or the outcome of pregnancy. They did not require any specific treatment. (Am J Obstet Gynecol 1991;165:51-6.)

Key words: Autoantibodies, antiphospholipids, hypertension in pregnancy

Antiphospholipid antibodies, the lupus anticoagulant and anticardiolipin antibodies, may be responsible for major complications of pregnancy; such as repeated early abortion,[1] second-trimester intrauterine fetal death, severe fetal growth retardation, and preeclampsia.[2-4] Antiphospholipid antibodies are often associated with organ-specific autoantibodies, and a reproductive autoimmune failure syndrome has been described.[5]

The prevalence of these autoantibodies has been well documented in normal pregnant women[6-8] and in pregnant women with early onset severe hypertension[9] but not during pregnancy complicated by gestational hypertension or idiopathic fetal growth retardation. The aim of this study was to evaluate the prevalence of antiphospholipid and other specific autoantibodies in pregnancies complicated by hypertension or idiopathic fetal growth retardation and to compare it with the prevalence of these autoantibodies in a general obstetric population.

Population and methods

Patients. Two hundred pregnant patients were screened for the presence of autoantibodies. The investigation was approved by the local Human Investigation Committee and informed consent was obtained from each patient. None of the patients had systemic lupus erythematosus or any other documented autoimmune disease such as sclerodermia, myasthenia, autoimmune thrombocytopenic purpura, or a history of thromboembolic disease. None of them was receiving chlorpromazine, which is known to induce lupus-like anticoagulants and autoantibodies.[10] All blood samples were taken during the third trimester of pregnancy, thereby excluding from the study early abortions and second-trimester intrauterine fetal deaths.

Normal pregnancies. One hundred patients without complications of pregnancy were enrolled during the period of the study from our antenatal clinic, and these formed the control population. All had normal pregnancies and none was receiving any registered medication.

Pregnancies complicated by idiopathic fetal growth retardation. Fifty patients had fetal growth retardation diagnosed during pregnancy by clinical examination and echography. Twenty-eight neonates had birth weight below the 10th percentile and 22 neonates had

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Table I. Populations and pregnancy outcome

	Hypertension (n = 50)	*Idiopathic fetal growth retardation (n = 50)*	*Normal pregnancy (n = 100)*
Age (yr)	29 ± 5.9	26 ± 5.4	27 ± 5.8
Parity	2.8 ± 1.5	2.2 ± 1.4	1.9 ± 1.6
Gestation at delivery (wk)	39 ± 1.8	38 ± 2.2	39 ± 2.1
Birth weight (gm)	2688 ± 583*	2386 ± 370*	3253 ± 265*
Birth weight <10th percentile (No.)	20 (40%)	28 (56%)	0
Birth weight <3rd percentile (No.)	8 (16%)	22 (44%)†	0
Fetal death (No.)	2 (4%)	0	0

$*p < 0.001$, compared with either of other two groups.
$\dagger p < 0.05$, compared with either of other two groups.

birth weight below the 3rd percentile. All patients had normal blood pressure. Three patients were taking 150 mg of aspirin daily (Juvépirine; Sarget, Mérignac, France) because of a history of severe fetal growth retardation during the previous pregnancy.

Pregnancies complicated by hypertension. In 50 patients high blood pressure developed during pregnancy. Twenty of these patients had moderate gestational hypertension (diastolic blood pressure <120 mm Hg),[11] five had severe gestational hypertension (diastolic blood pressure >120 mm Hg), 10 had late-onset moderate preeclampsia, five had early onset severe preeclampsia, two had superimposed preeclampsia, and eight had chronic hypertension, which was brought under control 3 months after delivery. All patients were on a regimen of oral antihypertensives: 26 received atenolol (Tenormine; Ici Pharma, Cergy, France), 10 were receiving methyldopa (Aldomet; Merck Sharp & Dohme Chibret, Paris), seven were receiving dihydralazine (Nepressol; Ciba-Geigy, Rueil-Malmaison, France), and one was taking clonidine (Catapressan; Boehringer-Ingelheim, Reims, France). Six patients were taking 150 mg of aspirin daily (Juvepirine; Sarget, Merignac, France) because of a history of severe hypertension during the previous pregnancy.

Clinical features and outcomes of pregnancy were compared in the two study populations and the control series (Table I). The medical histories of the multiparous women during the previous pregnancies were compared.

Methods

The 200 pregnant patients had standard coagulation tests, including prothrombin time, kaolin clotting time (activated partial thromboplastin time with kaolin as activator), thrombin time, assay of fibrinogen, antithrombin III activity and antigen, and protein C activity and antigen. Functional assay of antithrombin III and protein C was performed with specific chromogenic substrates (AT Prest and Stachrom Protein C-Stago; Diagnostica Stago, Asnières, France); an immunologic dosage of antithrombin III was given by laser nephelometry (Behring Diagnostic, Rueil-Malmaison, France).

Lupus anticoagulant. Antithromboplastin circulating anticoagulants were investigated, even when standard coagulation tests proved normal, by three additional procedures.

Tissue thromboplastin inhibition time. As described by Schleider et al.,[12] this test is based on the principle that antithromboplastin antibodies are more easily detected at low concentrations of phospholipids. The test was conducted with increasing dilutions of thromboplastin, from 1 : 100 to 1 : 4000. The ratio of the patient's plasma clotting time to the control plasma clotting time at a 1 : 100 dilution of thromboplastin was considered as abnormal when it exceeded 1.3.

Platelet neutralization procedure of Triplett et al.[13] This test implies adsorption of the patient's circulating anticoagulant by a platelet suspension. Shorter kaolin clotting time after adsorption compared with initial kaolin clotting time and a saline solution control, suggests the presence of a lupus-like inhibitor. Time reduction depends on the potency of the circulating anticoagulant; it was considered as significant if it exceeded 10 seconds when compared with initial kaolin clotting time.

Standard and high phospholipid concentration activated partial thromboplastin time. This procedure depends greatly on the detection of antithromboplastin antibodies in the reagent used. We used Platelin (Organon Teknika, Fresnes, France) as the source of phospholipids. This test is based on attenuation of the anticoagulant effect of antiphospholipid antibodies when phospholipids are added in excess. The ratio of the patient's clotting time to the control clotting time was established for two concentrations of platelin, a standard and a high concentration. The test result was considered positive when the patient/control ratio for the high concentration was lower than the patient/control ratio for the standard concentration and when the difference was >0.17.[14]

Anticardiolipin antibodies. Anticardiolipin antibodies were screened by the immunoenzymatic technique of Loizou et al.[15] For each serum sample the results were expressed as optical density corrected by comparison with an intralaboratory standard, tested during each procedure. A positive value was defined as an optical density absorbance >5 SD above the mean control value obtained in 107 normal subjects (107 blood donnors included as a control group).

The mean of this control group was estimated to be 0.11 optical density units (optical densities from 0.00 to 0.283), with a standard deviation of 0.07. Therefore, the value of the mean + 5 SD being 0.460 optical density units, the threshold of positivity for the study group was established at an optical density of 0.500 optical density units. All elevated samples underwent repeat testing and were checked against the standard serum of Harris and Hugues,[16] with a threshold of positivity above 15 GPL units (immunoglobulin G antiphospholipid units).

Autoantibody assays. Various antoantibodies against specific cell components were investigated.

Antinuclear antibodies were assayed by indirect immunofluorescence on slices of rat liver and smears of HEp2 cells. The different dilutions were incubated with substrate for 30 mintues at room temperature. The second antibody used was a fluorescein-conjugated goat polyvalent antiimmunoglobulin (anti-IgG, anti-IgA, anti-IgM, anti-κ, and anti-λ) (Institut Pasteur Production, Marne La Coquette, France). The results were given in serum dilutions. The threshold for positivity was established at 1:50, and any positive serum was then titrated by successive dilutions until extinction of fluorescence.

Anti–deoxyribonucleic acid antibodies were evaluated by indirect immunofluorescence on *Crithidia luciliae* cultures. Sera diluted 1:10 in phosphate buffer were incubated with substrate for 30 minutes at room temperature. A second fluorescein-conjugated goat polyvalent antibody (Institut Pasteur Production) allowed the detection of anti–deoxyribonucleic acid antibodies.

Antithyroglobulin antibodies were screened by passive hemagglutination (Thymune T; Wellcome, Paris). Antithyroid microsomal antibodies were assayed by passive hemagglutination (Thymune M; Wellcome).

Antiphospholipid antibodies may induce thrombocytopenia[2, 4] and false-positive syphilitic reactions[4]; platelet count was checked in all instances, and standard tests for syphilis (Venereal Disease Research Laboratory and *Treponema pallidum* hemagglutination) were done.

Statistical analysis. Variables between series were compared by the χ^2 test or by Yates' χ^2–derived test when the sample size was <5. Comparison of the means was done by analysis of variance. Results were expressed as means with standard deviations. Differences were considered as statistically significant for a p value <0.05.

Table II. Prevalence of autoantibodies

	Hypertension (n = 50)	*Idiopathic fetal growth retardation (n = 50)*	*Normal pregnancy (n = 100)*
Lupus anticoagulant	4	1	0
Anticardiolipin antibodies	2	0	0
Anti–cell component antibodies	2	0	3
TOTAL	16%*	2%	3%

*$p < 0.01$, compared with other two groups.

Results

Standard coagulation tests. Results of all 200 standard coagulation tests were normal, and no kaolin clotting time was >7 seconds above control, thereby eliminating the presence of frank lupus anticoagulant.

The prevalence of lupus anticoagulant, antiphospholipid antibodies, and cell component–specific autoantibodies is depicted in Table II for each series of patients.

Normal pregnancies. Among the 100 normal pregnancies, no lupus anticoagulant or anticardiolipin antibodies were found. The values obtained for anticardiolipin antibodies in this group of 100 normal pregnant women were equivalent to those established within the control group of 107 blood donors: a mean of 0.11 optical density units (for optical densities from 0.02 to 0.290) with a standard deviation of 0.07. Specific autoantibodies against cell components were detected in three patients: two had antithyroglobulin antibody at a dilution of 1:80 and one had antithyroid microsomal antibody at a dilution of 1:40.

Idiopathic fetal growth retardation. Of 50 patients with a growth-retarded fetus, none had anticardiolipin antibody or autoantibody against cell components. One patient with a small-for-gestational-age infant <3rd percentile had normal blood pressure, did not take aspirin, and had a false-positive syphilitic reaction. A lupus anticoagulant was detected by all three procedures (tissue thromboplastin inhibition time, at a dilution of 1:100, ratio of patient's clotting time/control clotting time of 1.48; platelet neutralization procedure, kaolin clotting times of 48.5 seconds initially and 35 seconds after platelet neutralization [saline solution control 43 seconds]; platelin neutralization test, standard – high ratio = 0.78).

Hypertension during pregnancy. Among the 50 pregnant patients with hypertension, eight had autoantibodies. Lupus anticoagulant was detected in four patients. One had positive results for all three proce-

Table III. Comparison of pregnancies with hypertension ($n = 50$) according to presence of autoantibodies

	Autoantibodies ($n = 8$)	*No autoantibodies ($n = 42$)*	*Significance*
Age (yr)	28 ± 5.3	29.4 ± 5.6	NS
Parity	2.6 ± 1.2	2.9 ± 1.3	NS
Birth weight (gm)	2670 ± 715	2548 ± 640	NS
Gestation at delivery (wk)	38.4 ± 1.9	39.6 ± 2.1	NS
Birth weight <10th percentile (No.)	3 (37%)	17 (40%)	NS
Birth weight <3rd percentile (No.)	0	8 (20%)	NS
Platelet count (No./mm^3)	286.000	283.000	NS

dures. For tissue thromboplastin inhibition, at a dilution of 1 : 100, the ratio of patient clotting time/control clotting time was 1.40. For the platelet neutralization procedure, initial kaolin clotting time was 39 seconds; after platelet neutralization it was 28 seconds; saline solution control was 37 seconds. For platelin neutralization test, difference standard − high ratio = 0.28. One patient had a positive platelet neutralization procedure (kaolin clotting time: initial, 47 seconds; after platelet neutralization, 37 seconds; saline solution control, 46 seconds). Two patients had positive results only for the platelin neutralization test (standard − high ratios of 0.35 and 0.40, respectively). All results were checked twice.

Anticardiolipin antibody was detected in two patients with optical density values of 0.600 and 0.733 optical density units, respectively. These results were duplicated for confirmation. The values found by checking against the standard serum of Harris and Hugues[16] were 17 and 20 GPL units, respectively.

Autoantibodies against cell components were found in three patients, two with antithyroid microsomal antibody ratios of 1 : 400 and 1 : 600, respectively, and one with weak antinuclear antibody dilution of 1 : 50.

The latter patient was one of the four with lupus anticoagulant; therefore a total of eight patients (16%) had autoantibodies detected among the pregnant patients with hypertension, including six (12%) with antiphospholipid antibodies. Both figures are significantly increased as compared with results in the control pregnant patients and the group with idiopathic fetal growth retardation ($p < 0.01$) (Table III).

Autoantibodies were equally distributed between patients with gestational hypertension (one with lupus anticoagulant, two with anticardiolipin antibodies, and one with antithyroid microsomal antibody) and those with preeclampsia (three with lupus anticoagulant and one with antithyroid microsomal antibody) (Table IV). The frequency of preeclampsia, gestational hypertension, and chronic hypertension, whether moderate or severe, was not different among the eight patients with hypertension and autoantibodies and the other 42 patients with hypertension but without autoantibodies (Table IV). The outcome of pregnancy also did not differ among the patients (Table III). The eight patients with hypertension and autoantibodies were multiparous, and the history of their previous pregnancies was compared with that of the 28 multiparous women with hypertension and autoimmune anomalies. A significantly more frequent occurrence of fetal growth retardation during previous pregnancies was recorded among patients with hypertension and autoantibodies (6/8 or 75% vs 8/28 or 28%, $p < 0.05$). A more frequent history of two or more spontaneous abortions also was noticed for these patients but the difference did not reach statistical significance (3/8 or 37% vs 4/28 or 14%, difference not significant).

None of the six patients with hypertension who received aspirin showed alterations in results of the coagulation tests. One patient receiving dihydralazine had lupus anticoagulant detected by the platelet neutralization procedure, but she did not have antinuclear or anti–deoxyribonucleic acid antibody. Six other patients receiving dihydralazine had no biologic autoimmune anomaly.

Comment

Although they act in vitro as anticoagulants, antiphospholipid antibodies induce in vivo procoagulant effects, interfering with the phospholipid antigens of prothrombin activators (activated factor X, factor V, and phospholipids).[2] More than 90% of patients with lupus anticoagulant also have anticardiolipin antibodies.[9] Despite recent evidence against their prostaglandin-mediated effects on cultured endothelium,[17] it has been suggested that antiphospholipid antibodies prevent prostacyclin production and cause hypertension.[18] They also bind to phospholipid antigens of the placental vessels, inducing placental infarction and fetal growth retardation or even fetal death.[1, 2, 19] Immunoglobulin G fractions from patients with antiphospholipid antibodies cause decidual necrosis and fetal death in mice.[20] Reproductive failures and obstetric complications have been well documented in patients with antiphospholipid antibodies,[1-4] and an antiphospholipid antibody syndrome has recently been proposed.[21]

In all previous studies[1-4, 6, 9, 22] lupus anticoagulant was

Table IV. Type and severity of hypertension according to presence of autoantibodies

	Autoantibodies (n = 8)	*No autoantibodies (n = 42)*	*Significance*
Moderate gestational hypertension	3 (1 LAC, 2 ACL) (37%)	17 (40%)	NS
Severe gestational hypertension	1 (1 ATM) (12%)	4 (9%)	NS
Moderate preeclampsia	2 (2 LAC) (25%)	8 (20%)	NS
Severe preeclampsia	1 (1 LAC) (12%)	4 (9%)	NS
Chronic hypertension	0	8 (20%)	NS
Superimposed preeclampsia	1 (1 ATM) (12%)	1 (2%)	NS

Moderate, Diastolic pressure <120 mm Hg; *severe,* diastolic pressure ≥120 mm Hg. *LAC,* Lupus anticoagulant; *ACL,* anticardiolipin antibody; *ATM,* antithyroid microsomal antibody.

assayed only by activated partial thromboplastin time and standard coagulation tests. The difference with our study was that for our 200 patients the standard coagulation test results and the kaolin clotting time were always normal. The sensitivity of activated partial thromboplastin time varies widely according to the methods used or the laboratory. It is possible that our standard coagulation tests lacked sensitivity, but the kaolin clotting time is usually considered to be very sensitive for the detection of lupus anticoagulants. We deliberately looked for a lupus anticoagulant with three additional procedures (tissue thromboplastin inhibition time, platelet neutralization procedure, standard and high phospholipid concentration activated partial thromboplastin time) even when the kaolin clotting time was normal. We found lupus anticoagulant in five patients despite a normal kaolin clotting time (four with hypertension-complicated pregnancies and one with growth retardation); therefore these procedures may be even more sensitive and detect lower levels of lupus anticoagulant or involve antigens of different specificity than the kaolin clotting time.

Pregnancies complicated by antiphospholipid antibodies share several common features with preeclampsia[9]; in both, the placenta may show infarctions and decidual vasculopathy. Indeed, lupus anticoagulant is often associated with preeclampsia.[2, 3] Two studies on the prevalence of antiphospholipid antibodies among preeclamptic patients resulted in conflicting conclusions. Scott[22] found no association between anticardiolipin antibodies and preeclampsia (of unspecified severity) whereas Branch et al.[9] showed significant levels of antiphospholipid antibodies in 16% of women with early onset severe preeclampsia. We also found that 16% of women with hypertension-complicated pregnancies had autoantibodies (12% had antiphospholipid and 4% had antithyroid microsomal antibodies). However, our population with pregnancy complicated by hypertension was not restricted to preeclampsia; some of the patients had gestational or chronic hypertension. In our study autoantibodies were equally distributed between patients with gestational hypertension and those with preeclampsia with three patients in each group having antiphospholipid antibodies and one patient in each group having antithyroid microsomal antibody (Table IV). Therefore investigation for antiphospholipid antibodies should not be limited to preeclampsia. Gleicher and El-Roeiy[5] also were able to demonstrate increased autoantibody levels in hypertensive diseases of pregnancy in comparison with nonpregnant controls. We confirmed their findings with a significantly higher prevalence of autoantibodies in pregnancy complicated by hypertension (16%) as compared with normal pregnancy (3%) ($p < 0.01$). However, whereas lupus anticoagulant may be responsible for major complications of pregnancy[2-4] and thromboembolic accidents,[9] in our study antiphopholipid antibodies were not related to an aggravation of hypertension (Table IV), an impairment of fetal growth, or a poor fetal outcome (Table III) among women with pregnancy complicated by hypertension. Therefore a need for treatment with heparin was not apparent.[23]

Lupus anticoagulant can induce intrauterine fetal growth retardation.[2] In our study the prevalence of lupus anticoagulant among pregnancies complicated by idiopathic fetal growth retardation was only 2%. However, in pregnancy complicated by hypertension, patients with autoantibodies had a significantly more frequent history of fetal growth retardation during a previous pregnancy than patients with no autoantibody (75% vs 28%, $p < 0.05$). Therefore only patients with pregnancy complicated by hypertension and a history of fetal growth retardation should be screened for autoantibodies.

In a general obstetric population the lupus anticoagulant detected by standard coagulation tests has a prevalence of 0.27%,[6] and there was a similar prevalence of 0.3% in a series of 1000 women with a history of fetal death or thromboembolic disease who did not have systemic lupus erythematosus.[24] In a series of 43 women with normal pregnancy El-Roeiy et al.[8] did not find lupus anticoagulant. In our series of 100 control pregnant women we did not find lupus anticoagulant, but it is possible that a larger sample might have yielded a small prevalence comparable to the findings of the above studies.

Anticardiolipin antibodies in two different low-risk obstetric populations had a prevalence of 2.2%[6] and 2.1%,[25] respectively. Again we did not find anticardiolipin antibody in our control population. This discrepancy may arise from a difference in sensitivity between the method of Loizou et al.[15] that we used and the immunoenzymatic methods of the other studies.

Autoimmune disorders result from polyclonal B lymphocyte activation; autoantibodies against cell components, such as antinuclear, anti–deoxyribonucleic acid, antithyroglobulin, or antithyroid microsomal antibodies, are often associated with antiphospholipid antibodies in unexplained infertility or early pregnancy loss.[5, 26] Evaluation of such autoantibodies during pregnancy has yielded conflicting results. Patton et al.[7] found a very high figure of 40.5% with autoantibodies among 84 normal pregnancies, including 25% with antinuclear and antithyroid microsomal antibodies, which is at odds with our findings. Conversely, El-Roeiy et al.[8] found that the majority of autoantibody levels in pregnant women were within normal nonpregnant ranges and that alterations occur, if at all, at the time of delivery. We found a 3% prevalence of antithyroid antibodies in our women with normal pregnancy. The difference with the study by El-Roeiy et al.[8] is that our patients were screened only once and only during the third trimester of pregnancy; therefore the potential influence of delivery on the level of autoantibodies was not examined.

We conclude that tests for the detection of autoantibodies during pregnancy should preferentially be performed for multiparous women with hypertension and a history of intrauterine fetal growth retardation (in addition to those with early onset severe preeclampsia). Although a specific treatment may not be needed when autoantibodies are present, a prolonged follow-up is required since they may foretell an autoimmune disease, the clinical manifestations of which may well appear several years later.[27]

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Automated analysis of near-term antepartum fetal heart rate in relation to fetal behavioral states: The Sonicaid System 8000*

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Fetal heart rate and fetal movements were recorded in 16 uncomplicated near-term pregnancies. The recordings were used to evaluate a system for automated fetal heart rate analysis (Sonicaid System 8000). Fetal rest-activity patterns were considered in the analysis. The mean duration of C2F periods "active sleep," 33 minutes) was significantly greater than that of C1F periods ("quiet sleep," 19 minutes) ($p < 0.001$). The incidence of accelerations and decelerations and the overall fetal heart rate variations were greater during C2F than during C1F ($p < 0.001$). In 11 of 16 C1F periods, the system classified the fetal heart rate variation as "questionable" or "abnormal." Episodes of high variation were identified in only 3 of 16 C1F periods, but they were found in all 18 C2F periods. Episodes of low variation were identified in 14 of the 16 C1F periods but were not found in any C2F periods. During C2F periods, the system's criteria of normality were met in all cases but one; they were not met during any of the C1F periods. Thus the diagnosis of fetal distress should not be based merely on the absence of accelerations, low fetal heart rate variation, or absence of episodes of high variation in recordings with a duration of <45 minutes. (AM J OBSTET GYNECOL 1991;165:57-65.)

Key words: Automated analysis, antepartum fetal heart rate, fetal heart rate monitoring, fetal behavioral states, fetal rest-activity patterns, fetal movements

Antepartum fetal heart rate (FHR) monitoring is widely applied to assess the fetal condition in high-risk pregnancies. However, visual interpretation of antepartum FHR recordings is hampered by poor intraobserver and interobserver agreement.[1-3] This problem might be reduced with the help of automated FHR analysis. Such analyses would offer the obstetrician objective quantitative information on FHR parameters, including trends that occur during days to weeks.

For several years we have used a computerized system[4] to study the relationship between fetal movements, which were observed via real-time ultrasonography, and FHR. Four behavioral states have been described in the near-term fetus. These behavioral states are based on three variables: the FHR pattern, fetal body movements, and fetal eye movements.[5] The behavioral state analysis is performed semiautomatically: FHR patterns must be classified initially by visual inspection of the recordings.

Recently, a system for automated antenatal FHR analysis (Sonicaid System 8000) has become commercially available. This study evaluates how this system performs with respect to FHR recordings obtained during various fetal behavioral states.

Material and methods

Recordings. Sixteen recordings of antepartum FHR and fetal movements were obtained in uncomplicated pregnancies. Gestational age at the time of the recording (38 to 39 weeks) was confirmed by early ultrasonographic examination.

All infants were born around term and had birth weights that were appropriate for gestational age, Apgar scores of 10 at 5 minutes, and a pH of >7.20 in the umbilical artery. All infants appeared to be normal on neurologic examination.

FHR and fetal movements were recorded continuously for 2 hours. The participating women were asked not to smoke and not to drink coffee, tea, or alcoholic beverages in the 2 hours before the recording was done. The recordings took place during the afternoon. The women were in a semirecumbent position and were slightly tilted to the left. Fetal movements were observed by means of two real-time ultrasonographic units. One examiner directed the transducer to the fetal head to observe eye, mouth, and head movements. A second examiner directed the transducer to the fetal trunk so that trunk and arm movements, breathing movements, and hiccups and startles could be ob-

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**Sonicaid System 8000 is a registered trademark of Oxford Sonicaid Ltd., Chichester, Great Britain.*
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Table I. Coincidence of behavioral state parameters*

Period	*Eye movements*	*Body movements*†	*FHR pattern (type and description)*
C1F	Absent	Incidental	A: Stable FHR pattern, small oscillation bandwidth, possible isolated accelerations
C2F	Present	Periodic	B: FHR pattern with wider oscillation bandwidth than FHR pattern A, frequent accelerations
C3F	Present	Absent	C: Stable FHR pattern, wider oscillation bandwidth than FHR pattern A, no accelerations
C4F	Present	Continuous	D: Unstable FHR pattern, large and long-lasting accelerations or tachycardia

C1F, Coincidence of parameters that match state 1F; *C2F*, coincidence of parameters that match state 2F; *C3F*, coincidence of parameters that match state 3F; *C4F*, coincidence of patterns that match state 4F.

*Criteria of Nijhuis et al.[5]

†Trunk, head, or arm movements.

served. Movements were recorded on line with coded event markers. Both examiners used a control device to indicate adequate visibility of the fetal orbit and trunk.[4, 6]

FHR was recorded by means of wide-range ultrasonographic equipment (HP 8040A, Hewlett Packard Co., Palo Alto). The FHR monitor supplied an analog signal that was sampled at a rate of 10 times per second and was converted from analog to digital. The sample values were stored in a computer (PDP 11/34, Digital Equipment B.V., Utrecht, The Netherlands) with the information on fetal movements.[4]

Fetal behavioral states. According to Nijhuis et al.,[5] the determination of fetal behavioral states is based on particular FHR patterns and the presence or absence of fetal eye and body movements (which consist of trunk, arm, and head movements).

The presence or absence of fetal eye and body movements was assessed via an extended automated window procedure including correction for signal loss that resulted from inadequate visibility of the fetal orbit or trunk.[4]

A Delphi group opinion procedure was applied to classify FHR patterns.[6] According to the fetal behavioral state concept, four different FHR patterns can be distinguished (Table I).[5]

Automated assessment of fetal behavioral states was performed by combining the results of the Delphi procedure (FHR patterns) and the automatic window procedure (fetal eye and body movements). Specific combinations of the parameters of body movements (absence or presence), eye movements (absence or presence), and FHR patterns (A through D) match the four fetal behavioral states (1F to 4F).[5] These combinations of state parameters, which are called coincidence 1F through coincidence 4F (C1F through C4F), are summarized in Table I. An example of a recording of FHR and fetal movements and the results of the behavioral state analysis are given in Fig. 1.

The System 8000. The System 8000 collects information on FHR, tocodynamometry, and maternal perception of fetal movements.[7-12] Data acquisition proceeds continuously up to a maximum of 60 minutes. Analysis is performed after 10 minutes and every 2 minutes thereafter. If the record appears to be normal according to the system's criteria, the computer displays *Dawes/Redman criteria met,* in which case the recording may be stopped. Otherwise, the advice *continue* is given.

In the analysis procedure the computer fits a baseline to the trace, identifies accelerations and decelerations, and calculates the FHR range in milliseconds for each minute of the trace. The computer marks episodes of high and low variation and measures basal heart rate.

Accelerations are defined as periods with an amplitude >10 beats/min above baseline and a duration >15 seconds. Criteria for decelerations are amplitude >10 beats/min below baseline and duration >1 minute or amplitude >20 beats/min below baseline and duration >30 seconds. A deceleration with an area >20 lost beats is considered large.

The overall long-term FHR variation is expressed as the mean minute range (in milliseconds), which is calculated as the average of the differences between minimum and maximum pulse intervals in each minute, unless (as during a long acceleration) all pulse intervals are less than baseline, in which case the range is measured from baseline. An overall variation >30 milliseconds is classified as normal; between 20 and 30 milliseconds, as questionable; and <20 milliseconds, as abnormal. Any minute of the trace that contains a deceleration is excluded from the calculation of the overall variation and from episodes of high variation.

An episode of high variation is a section of the trace in which at least five of every six consecutive minutes have a mean minute range that is above a certain threshold. Similarly, in an episode of low variation, five of six consecutive minutes have a mean minute range that is below a certain threshold. At the start or end of

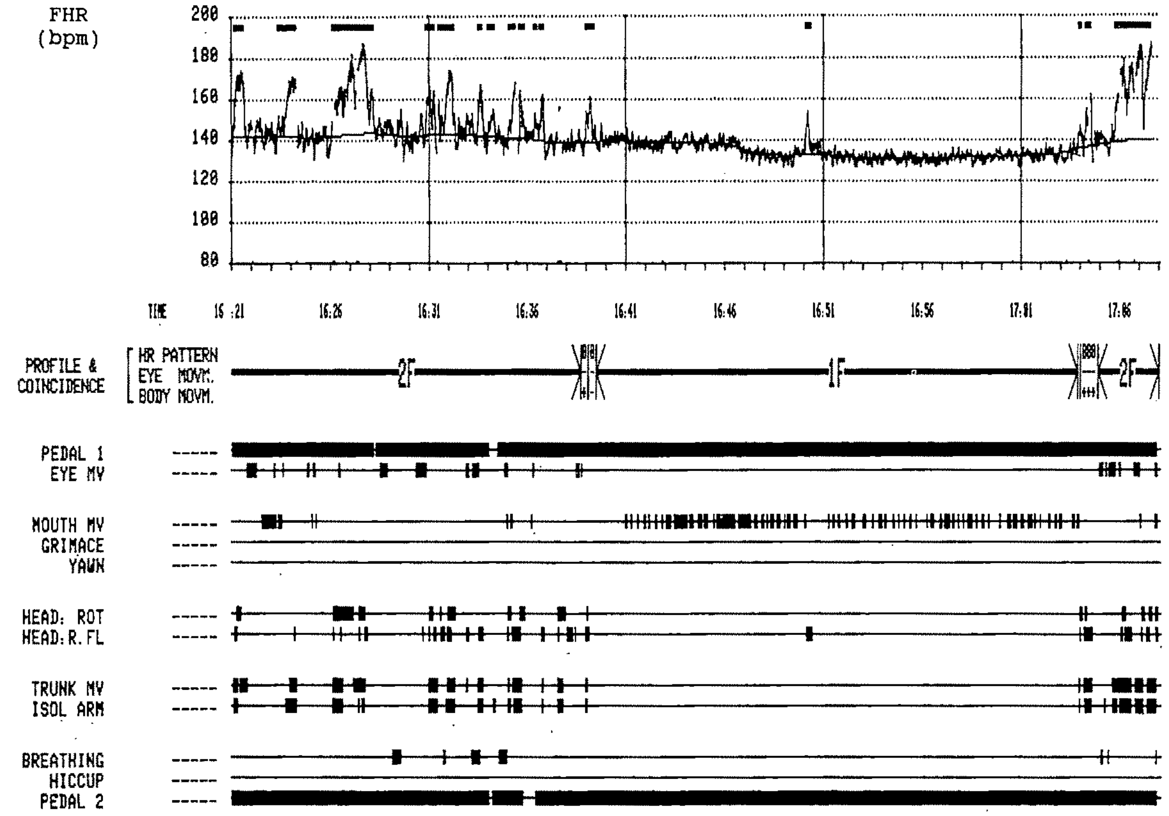


Fig. 1. Example of recording of FHR, fetal movements *(MV)*, and results of behavioral state analysis. *Top*, Automated FHR analysis according to Mantel et al.[17, 18] Accelerations are indicated by *thick solid line*. *Bottom*, Fetal movements *(MV)*. *Pedals 1 and 2* indicate adequate visibility of fetal orbit and trunk. *ROT*, Rotation; *R.FL*, retroflexion. *Middle*, Result of automated window procedure with two C2F periods (16:21:00 to 16:38:45 and 17:05:00 to 17:08:00) and one C1F period (16:39:30 to 17:03:55) between these.

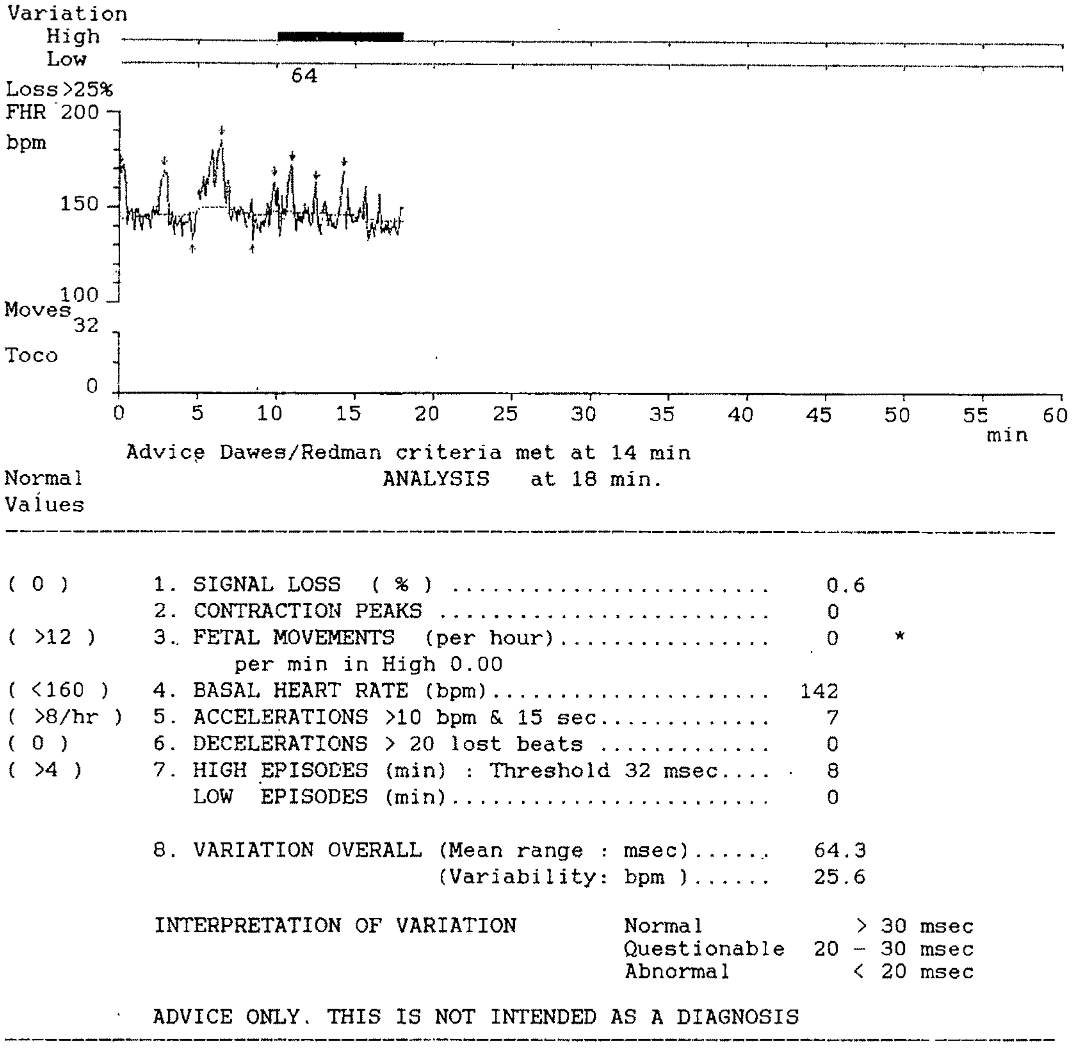
a record, the computer will accept 4 of 5 minutes as an episode of high variation or an episode of low variation. On the first analysis, the thresholds are 32 milliseconds for high variation and 30 milliseconds for low variation. If the program has not found an episode of high variation after 20 minutes of FHR recording, it reduces the thresholds to 24 and 22 milliseconds, respectively, and performs a second analysis.

Basal heart rate is defined as the mean heart rate averaged during all episodes of low variation. If no episode of low variation is present, the basal heart rate is derived from a statistical analysis of the frequency distribution of pulse intervals.

The statement *Dawes/Redman criteria met* is given as soon as the following three requirements have been fulfilled: presence of an episode of high variation, identification of at least one fetal movement (from maternal perception) or of three accelerations, and absence of large decelerations.

Automated analysis of FHR in relation to fetal behavioral states. To evaluate the System 8000 in relation to fetal behavioral states, all periods C1F through C4F with a minimum duration of 10 minutes were selected from the 16 recordings. FHR signals of these periods were read from disks, were converted from digital to analog, and were sent real-time to the fetal heart rate monitor (HP 8040A, Hewlett Packard), which was connected to the System 8000. Information on uterine activity or maternal perception of fetal movements was not part of this study.

The following parameters were analyzed for each period C1F through C4F: duration (minutes), signal loss (percent), basal heart rate (beats per minute), number of accelerations and decelerations per hour, total duration of episodes of high variation and episodes of low variation (minutes and percents), overall mean heart rate variation (milliseconds), and mean heart rate variation during episodes of high variation and episodes of low variation. Finally, we evaluated whether the appropriate criteria were met and, if so, after how long and at which threshold. Statistical comparison between different periods of coincidence was performed



```
            Advice Dawes/Redman criteria met at 14 min
Normal                         ANALYSIS    at 18 min.
Values
--------------------------------------------------------------------------------

( 0 )       1. SIGNAL LOSS  ( % ) ........................   0.6
            2. CONTRACTION PEAKS ..........................   0
( >12 )     3. FETAL MOVEMENTS  (per hour)................   0      *
                  per min in High 0.00
( <160 )    4. BASAL HEART RATE (bpm).....................  142
( >8/hr )   5. ACCELERATIONS >10 bpm & 15 sec.............   7
( 0 )       6. DECELERATIONS > 20 lost beats .............   0
( >4 )      7. HIGH EPISODES (min) : Threshold 32 msec....   8
               LOW  EPISODES (min)........................   0

            8. VARIATION OVERALL (Mean range : msec)......  64.3
                                 (Variability: bpm )......  25.6

            INTERPRETATION OF VARIATION        Normal          > 30 msec
                                               Questionable 20 - 30 msec
                                               Abnormal        < 20 msec

            ADVICE ONLY. THIS IS NOT INTENDED AS A DIAGNOSIS
--------------------------------------------------------------------------------
```

Fig. 2. Automated analysis by System 8000. Results of exclusive analysis of first C2F period as shown in Fig. 1 (16:21:00 to 16:39:00). See text for explanation.

by analysis of variance with $p < 0.05$ used as the threshold of significance.

Results

In the 16 recordings, the following numbers of periods C1F through C4F with a duration >10 minutes were identified: C1F, 16 (12 records with one C1F period, two records with two C1F periods); C2F, 18 (14 records with one C2F period, two records with two C2F periods); C3F, 2 (two records); C4F, 4 (four records). As a result of the small numbers for C3F and C4F, further analysis was restricted to C1F and C2F. Figs. 2 and 3 show examples of the exclusive FHR analysis of C2F and C1F periods, respectively, by the System 8000.

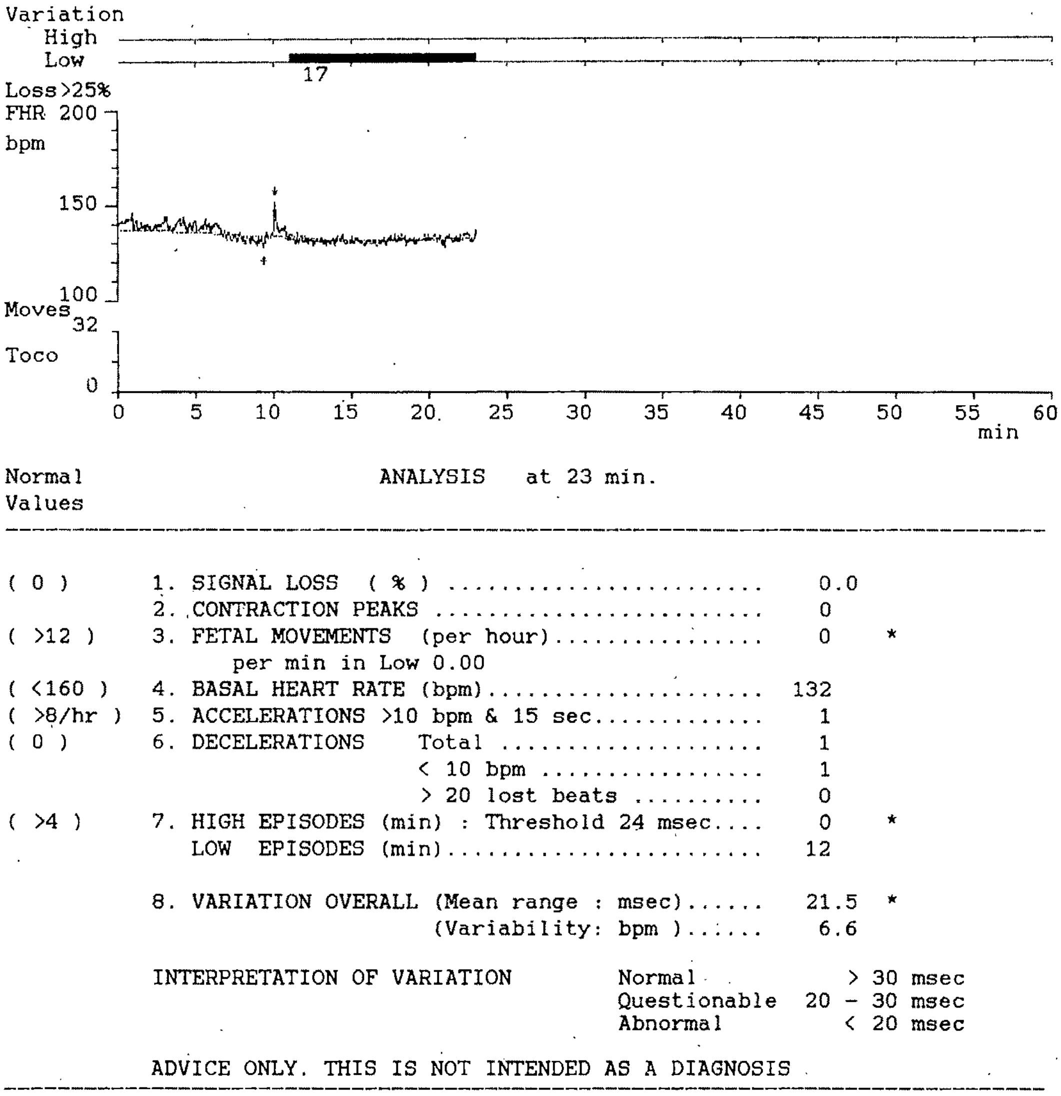
The mean duration (19 minutes) of C1F periods (95% confidence interval, 15 to 22; range, 11 to 34) was significantly less than that of C2F periods, which was 33 minutes (95% confidence interval, 26 to 41; range, 10 to 60) ($p < 0.001$).

Mean signal loss during C1F periods was 1.2% (95% confidence interval, 0.4 to 2.0; range, 0.0 to 4.8), compared with 3.7% during C2F periods (95% confidence interval, 2.0 to 5.4; range, 0.4 to 10.0) ($p < 0.01$).

Mean basal heart rate during C1F was 136 beats/min (range, 120 to 143), compared with 137 beats/min during C2F (range, 125 to 146) ($p = 0.44$).

The mean number of accelerations per hour totaled 3.4 during C1F (95% confidence interval, 1.4 to 5.4; range, 0 to 10) and 21.5 during C2F (95% confidence interval, 17.7 to 25.4; range, 6 to 38) ($p < 0.001$).

Only 2 of 16 C1F periods contained a deceleration. In contrast, 9 of 18 C2F periods contained one or more decelerations (range, 1 to 5). This difference is statistically significant if the differences in duration of pe-



```
Normal                  ANALYSIS   at 23 min.
Values
------------------------------------------------------------------------

( 0 )      1. SIGNAL LOSS  ( % ) .......................    0.0
           2. CONTRACTION PEAKS .........................    0
( >12 )    3. FETAL MOVEMENTS  (per hour)................    0     *
                per min in Low 0.00
( <160 )   4. BASAL HEART RATE (bpm)....................  132
( >8/hr )  5. ACCELERATIONS >10 bpm & 15 sec............    1
( 0 )      6. DECELERATIONS      Total ..................    1
                                 < 10 bpm ...............    1
                                 > 20 lost beats ........    0
( >4 )     7. HIGH EPISODES (min) : Threshold 24 msec....    0     *
              LOW  EPISODES (min)........................   12

           8. VARIATION OVERALL (Mean range : msec)......   21.5   *
                                (Variability: bpm )......    6.6

           INTERPRETATION OF VARIATION      Normal          > 30 msec
                                            Questionable  20 - 30 msec
                                            Abnormal        < 20 msec

           ADVICE ONLY. THIS IS NOT INTENDED AS A DIAGNOSIS
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```

Fig. 3. Automated analysis by System 8000. Results of exclusive analysis of C1F period as shown in Fig. 1 (16:40:00 to 17:03:00). See text for explanation.

riods C1F and C2F are considered ($p < 0.001$). However, these decelerations were of small magnitude (area <20 lost beats).

During the first analysis, episodes of high variation (threshold, 32 milliseconds) were identified in only 2 of the 16 C1F periods. In both of these cases there was a shift in basal heart rate that caused a deviation from the less tractable baseline and thereby enabled identification of an episode of high variation. In all the remaining 14 cases only episodes of low variation were identified. In 7 of these 14 cases the duration of the C1F period was ≥20 minutes, in which case a second analysis was performed. This resulted in recognition of an additional episode of high variation (threshold, 24 milliseconds) in only one case. The analysis of the 14 C1F periods with episodes of low variation showed that 64% of the time during C1F (range, 25% to 100%) was spent in episodes of low variation (mean duration, 12.4 minutes; range, 5 to 30 minutes). Episodes of low variation were not found in any of the 18 C2F periods. In contrast, episodes of high variation were identified in all 18 C2F periods during the first analysis. On average, 72% of the time during C2F (range, 20% to 100%) was spent in episodes of high variation (mean duration, 23.9 minutes, range, 5 to 41 minutes).

The Dawes/Redman criteria were not met during any of the 16 C1F periods, mostly because of absence of an episode of high variation (13 cases). In the three C1F periods in which an episode of high variation was found, the criteria were not met because fewer than

Table II. Quantitative data of automated FHR analysis of C1F and C2F

	C1F (n = 16)			*C2F (n = 18)*		
	Mean	*95% Confidence interval*	*Range*	*Mean*	*95% Confidence interval*	*Range*
Duration (minutes)	19	15-22	11-34	33	26-41	10-60
Sign loss (%)	1.2	0.4-2.0	0-4.8	3.7	2.0-5.4	0.4-10
Basal fetal heart rate (beats/min)	136	—	120-143	137	—	125-146
Accelerations (No./hr)	3.4	1.4-5.4	0-10	21.5	17.7-25.4	6-38
FHR variation* (milliseconds)						
Overall	25.7	22-29	14-36	56.3	50-63	40-82
During ELV	18.7†	—	13-27†	—	—	—
During EHV	38.4‡	—	32-46‡	58.3	—	41-82

The results concern 16 C1F periods (14 recordings) and 18 C2F periods (16 recordings), unless stated otherwise.

*Expressed as mean minute range, overall and exclusively during episodes of low variation *(ELV)* or high variation *(EHV)*.

†Fourteen C1F periods.

‡Three C1F periods.

three accelerations were identified. During the analyses of the 18 C2F periods, the Dawes/Redman criteria were met after 10 minutes in 12 cases, 12 minutes in 2 cases, 14 minutes in 2 cases, and 18 minutes in 1 case. The statement did not appear in one C2F period (duration, 10 minutes) because only one acceleration was identified.

The overall mean FHR variation during C1F was 25.7 milliseconds (95% confidence interval, 22 to 29; range, 14 to 36). The overall FHR variation was >30 milliseconds in 5 of 16 cases, between 20 and 30 milliseconds in 7 of 16 cases, and <20 milliseconds in the remaining four C1F periods. The lowest value (13.5 milliseconds) was found during a C1F period that lasted 22 minutes. The longest C1F period (34 minutes) had an overall mean FHR variation of 19.9 milliseconds. The overall mean FHR variation during C2F was 56.3 milliseconds (95% confidence interval, 50 to 63; range, 40 to 82), which is significantly more than during C1F ($p < 0.001$). Expressed in beats per minute instead of in milliseconds, the overall mean FHR variability was 8.6 beats/min during C1F (95% confidence interval, 7 to 9; range, 5 to 11), compared with 20.3 beats/min during C2F (95% confidence interval, 18 to 23; range, 14 to 32).

During episodes of low variation within C1F periods, which were noted in 14 of 16 cases, the mean minute range of variation varied between 13 and 27 milliseconds (average value, 18.7 milliseconds). During episodes of high variation within C1F periods, which were noted in 3 of 16 cases, this range was 32 to 46 milliseconds. In contrast, during episodes of high variation within periods C2F, which were noted in all 18 cases, the mean minute range of variation varied between 41 and 82 milliseconds (average value, 58.3 milliseconds).

The results are summarized in Table II.

Comment

In near-term recordings of 2 hours' duration, the fetus spends about 25% of the time in C1F ("quiet sleep") and about 50% in C2F ("active sleep"). The majority of remaining time is spent without coincidence of state parameters. C3F and C4F are relatively rare.[6]

The significantly shorter duration of C1F compared with that of C2F (mean values in current study, 19 vs 33 minutes) does not seem to be of great clinical importance but is discussed in greater detail at the end of this section. In reality, the difference in mean duration will even be greater because C2F periods may last for >90 minutes. In this study C2F periods could be included only up to a maximum of 60 minutes, which is the limit that the System 8000 can handle.

The significantly lower amount of signal loss during C1F (mean, 1.2%) compared with that of C2F (mean, 3.7%) corresponds to that of the literature.[8, 10] It is unlikely that this amount of signal loss could have caused the other differences found between C1F and C2F.

Lack of difference in mean basal heart rate between C1F and C2F relates to the methods in use and is difficult to interpret. Basal heart rate is defined by the System 8000 as mean rate averaged over all periods recognized as episodes of low variation. If episodes of low variation are absent, basal heart rate is derived from a statistical analysis of the frequency distribution of pulse intervals.[9] In this study no episodes of low variation were found during any of the 18 C2F periods, whereas episodes of low variation were detected in 14 of the 16 C1F periods.

The higher incidence of accelerations during C2F (mean, 21.5 per hour) compared with that of C1F (mean, 3.4 per hour) is hardly surprising because the frequency of accelerations is part of the definition of the FHR patterns (Table I). A strong association between fetal movements and accelerations in uncomplicated pregnancies is well known from the literature.[5, 7, 13-15] A similar program for automated FHR analysis (without consideration of fetal rest-activity patterns) has been used before.[10, 16] Visser et al.[16] found a mean incidence of accelerations of 14.0 per hour at 37 to 38 weeks of gestation and 15.0 per hour at 39 to 40 weeks of gestation. At 38 to 39 weeks of gestation, Dawes et al.[10] reported an overall incidence of 15.5 accelerations per hour, compared with 21.6 per hour when analyzed exclusively during episodes of high variation. It should be noted that the latter value is almost the same as that found during C2F in this study.

C2F periods more often contained decelerations than did C1F periods (50% vs 12.5%). These decelerations were small (area <20 lost beats). Most of the decelerations during C2F were either identified immediately after accelerations[7, 10, 13] or between groups of accelerations.[17, 18]

The statement *Dawes/Redman criteria met* did not appear during any of the C1F periods, but the criteria were met in 17 of 18 C2F periods. The one C2F period during which the criteria were not fulfilled contained a downward shift in basal heart rate, which caused a too-high baseline fit and resulted in the identification of only one acceleration. If perception of fetal movements had been part of this study, the criteria would probably have been met because this C2F period contained several body movements.

The strong correlation between fetal rest-activity patterns and FHR is reflected not only by the incidence of accelerations but also by the FHR variation itself. The overall mean minute range of variation during C1F (range, 14 to 36 milliseconds) does not show an overlap with that during C2F (range, 40 to 82 milliseconds). Again, this is no surprise when the definitions of the FHR patterns A and B are considered. During episodes of low variation within C1F periods, the mean minute range of variation was 18.7 milliseconds on average, compared with 58.3 milliseconds during episodes of high variation within C2F periods. These values are comparable to those found during episodes of high variation and episodes of low variation in uncomplicated near-term pregnancies when fetal rest-activity cycles were not considered.[12]

Three studies, in which a precursor of the System 8000 was used to study FHR in complicated pregnancies, will be discussed in more detail. Henson et al.[19] studied 1-hour FHR recordings of mainly growth-retarded fetuses who were delivered between 28 and 37 weeks of gestation because of antenatal fetal compromise. The overall mean minute range of variation (average value, 22.9; range, 13 to 34 milliseconds), was significantly lower when compared with that of normal pregnancies of the same gestational age.[19] The values found in the compromised group, however, are comparable to those found during C1F in this study.

A second study by the same authors indicated that growth-retarded fetuses with reduced FHR variation show rest-activity patterns similar to those of normal fetuses but that the changes in variation are of lower amplitude.[20] Within a subgroup of fetuses with the lowest variation, the mean minute range during episodes of low variation was 17.5 milliseconds, on average. In this study of uncomplicated pregnancies the mean minute range within episodes of low variation during C1F was 13 to 27 milliseconds. Both studies stress the necessity of accounting for fetal rest-activity patterns when antenatal FHR recordings are interpreted.

Smith et al.[21] studied antenatal FHR variation in relation to the respiratory and metabolic status of the compromised human fetus. The fetuses were delivered between 28 and 36 weeks of gestation because of fetal distress as judged from conventional 1-hour FHR recordings. The automated analysis of these recordings showed a significantly lower overall FHR variation when compared with the recordings of the control group. The reduced variation (average mean minute range, 20.6 milliseconds) appeared to be associated with fetal hypoxemia but not with asphyxia, which is consistent with earlier observations.[19, 22, 23] Smith et al.[21] concluded that measurement of FHR variation over 60 minutes can help to identify fetuses who are becoming hypoxemic without acidemia, that a mean minute range <20 milliseconds should be considered abnormal, and that a mean minute range between 20 and 30 milliseconds should be considered probably abnormal.

This study of uncomplicated pregnancies, however, showed that 7 of 16 C1F periods had a mean overall FHR variation between 20 and 30 milliseconds, which, according to the system's classification, should be considered questionable. In addition, there were four cases with an overall FHR variation <20 milliseconds, which should be considered abnormal. However, because these C1F periods had a duration <60 minutes, the system advised continuing the recording at the end of these periods. In this case a period of higher variation would have been entered, which almost certainly would have resulted in a mean minute range above the given threshold for abnormality. The duration of C1F periods is therefore one of the most important characteristics to consider if the results of this study are compared with those found in compromised fetuses by Henson et al.[19, 20] and by Smith et al.[21] A low FHR variation should not be considered abnormal without consideration of the duration of the recording, not even if the mean minute range is as low as 13.5 milliseconds (about 5 beats/min), which was the lowest value found during C1F in the present study.

The key question is, how long do these periods of fetal rest last in uncomplicated pregnancies? In 24-hour studies at 37 to 39 weeks of gestation, Visser et al.[24] found a mean duration of episodes of low variation of 17.1 minutes (range, 6 to 38 minutes). The percentage of time spent in low variation was subject to individual as well as diurnal variation, but there was no diurnal variation in the length of episodes of low variation.[24] Patrick et al.[25] reported the longest period of complete absence of gross fetal body movements to be 50 minutes near term (isolated movements of the extremities were not included). If fetal rest is defined as a time interval containing fewer than three consecutive accelerations, then the longest period of fetal rest was 82 minutes near term.[26] The answer to the question depends on the chosen criteria for "fetal rest." In this study the fetal behavioral state concept was used as the gold standard to determine rest-activity patterns. The longest C1F period had a duration of 34 minutes. From earlier studies, we know that C1F periods can last for 40 minutes near term, although FHR pattern A may last for 45 minutes both near term and at a gestational age of 32 weeks (unpublished data). In a recent study of uncomplicated pregnancies, basal heart rate variability was measured as bandwidth over successive 30-second intervals between accelerations. There appeared to be no significant difference in mean bandwidth during C1F at 32 weeks when compared with that at 38 weeks' gestational age (unpublished observations).

As a result of this study and results known from the literature, we concluded that the automated analysis of FHR patterns during C1F and C2F periods strongly (although indirectly) supports the association between FHR patterns and rest-activity states in utero. The criteria for normality that are used by the System 8000 are defined so that they are almost always fulfilled whenever a period of C2F with a duration of longer than 10 minutes is recorded. We furthermore conclude that a diagnosis of fetal distress should not be determined merely on the basis of absence of accelerations, a low overall mean minute range of FHR variation, or on the absence of periods of high variation if the recording did not last >45 minutes. Obstetricians should be aware that analysis of FHR recordings of compromised fetuses may give results that are comparable to those found in uncomplicated pregnancies during C1F. The advice of the system, which is to continue recording until the criteria are met (if necessary, for as long as 60 minutes), may prevent errors in the interpretation of antepartum FHR recordings.

We thank Mrs. E.E. van Woerden, MD, for placing the recordings of FHR and fetal movements at our disposal. We are grateful to H.C. Schoemaker, MSc, for his valuable advice concerning the statistical analysis of this study.

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A life-threatening sexually transmitted *Haemophilus influenzae* in septic abortion: A case report

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Haemophilus influenzae infections of the genitourinary tract are rare. A case of a life-threatening *Haemophilus influenzae* bacteremia associated with a septic abortion is presented. Sexual transmission of bacteria after orogenital contact is proposed as a possible source of this uncommon infection. (AM J OBSTET GYNECOL 1991;165:66.)

Key words: *Haemophilus influenzae*, septic abortion

Haemophilus influenzae infections are uncommon in adults, usually manifested as pneumonia or bronchitis. Although genitourinary tract *H. influenzae* infections are rare, tuboovarian abscess, salpingitis, and urinary tract infections have been reported. *H. influenzae* in septic abortion was first described in 1973.[1] Since then only a few cases have been reported, generally characterized by mild maternal course (i.e., a virtual absence of symptoms or low-grade fever). Most of these women did not need treatment. Recently, however, we treated a patient with hypotension, shock, and disseminated intravascular coagulation as a result of *H. influenzae* infection associated with septic abortion.

Case report

A 28-year-old gravida 3, para 1 woman whose pregnancy had been uneventful up to 13 weeks' gestation was admitted to our department because of vaginal bleeding and high fever (up to 40° C) of 36 hours' duration. Results of a physical examination were normal and vaginal inspection revealed a dilated cervical os and amniotic fluid leakage. Broad-spectrum antibiotic therapy was administered and uterine curettage was performed after induction of general anesthesia. A few hours later high fever, chills, hypotension, oliguria, and confusion developed in the patient. Laboratory findings revealed leukopenia (1800/mm^3), anemia (9.3 gm/dl), thrombocytopenia (60,000/mm^3), prolonged partial thromboplastin time (60 seconds), abnormal prothrombin time (25%), elevated fibrinogen split-products level (400 mg/dl), elevated levels of liver enzymes (serum glutamate oxaloacetate transaminase 215 U/L, serum glutamate pyruvate transaminase 163 U/L), bilirubin (4.6 mg/dl), and compensated metabolic acidosis. Chest roentgenogram was normal. The patient was admitted to the intensive care unit and required therapy with intravenous dopamine and blood-product transfusions. Nontypable *H. influenzae* was isolated in blood cultures and antibiotic treatment was administered according to bacterial sensitivity.

Comment

H. influenzae is only rarely a vaginal commensal organism, accounting for <1% of bacterial growth. Recently, an increasing incidence of *H. influenzae* neonatal sepsis has been reported.[2] Sexually transmitted *H. influenzae* after orogenital contact has been proposed as a possible source of urogenital infection.[1] Our patient reported orogenital sexual contact approximately 72 hours before the onset of symptoms. Thus it seems that the incidence of *H. influenzae* urogenital infection may be affected by changes in sexual behavior, as has been noted in infections with other throat microorganisms. In contrast with the mild clinical disease in previously reported cases of *H. influenzae*–infected abortions or *H. influenzae* peripartum infections, this case warns that *H. influenzae* infection associated with septic abortion may run a severe, life-threatening course.

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The association between human papillomavirus deoxyribonucleic acid status and the results of cytologic rescreening tests in young, sexually active women

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We examined the utility of cytologic rescreening tests in women who had positive test results for human papillomavirus deoxyribonucleic acid but who were diagnosed as having benign conditions at cytologic testing. One hundred twenty-five Papanicolaou smears from women who were screened for human papillomavirus deoxyribonucleic acid were sent routinely to a private laboratory for diagnoses. These slides were then reviewed independently by two pathologists who were blinded to the human papillomavirus deoxyribonucleic acid results. The effects of cytologic rescreening in cases of both positive and negative human papillomavirus deoxyribonucleic acid were assessed by calculating *z* scores. Cervical intraepithelial neoplasia was diagnosed in 40% by pathologist A and in 20% by pathologist B of the human papillomavirus–positive subjects compared with none diagnosed by the private cytology laboratory ($z = 3.09$, $p < 0.005$ and $z = 1.98$, $p < 0.05$, respectively). No significant differences were found in the human papillomavirus–negative group. We conclude that cytologic rescreening in human papillomavirus deoxyribonucleic acid–positive women who were initially diagnosed as having benign cytologic results will yield a significant proportion of cases of cervical intraepithelial neoplasia. (AM J OBSTET GYNECOL 1991;165:67-71.)

Key words: Human papillomavirus, cytology, rescreening

The strong association between cervical neoplasia and human papillomavirus (HPV) infection[1, 2] has stimulated interest in HPV diagnostic tests. Currently, the Papanicolaou smear is used as the primary screening test for cervical disease, including condyloma and intraepithelial neoplasia.[3] However, the Papanicolaou smear appears to be relatively insensitive, detecting only 50% to 80% of the abnormalities found by histologic examination of cervical biopsy specimens obtained by colposcopy.[4-6] Routine colposcopy remains a time-consuming and cost-ineffective means to identify cervical disease. Therefore tests that identify HPV deoxyribonucleic acid (DNA) have been developed in the hope that these techniques may augment our ability to precisely identify women who are infected with HPV and who may be at risk for cervical intraepithelial neoplasia.[7] The indications for and clinical utility of these DNA detection tests have not yet been established. However, the correlation between HPV DNA status and cytologic abnormalities is not absolute[6]; i.e., some women with abnormal Papanicolaou smears will not have detectable HPV DNA and, conversely, many patients with detectable HPV DNA appear to have normal Papanicolaou smears. Therefore, whereas HPV detection tests could augment cytologic testing, they are not equivalent replacements.

It has been suggested that HPV DNA testing may be used to identify women at high risk for cervical intraepithelial neoplasia. Previous studies have reported that when cytologic smears from women who are HPV positive and who have "normal" cytologic results are reviewed additional cytologic abnormalities (including intraepithelial neoplasia) can be detected.[8, 9] These findings seem to indicate that the combined use of HPV DNA testing and the Papanicolaou smear might improve the identification of women with cervical disease. Unfortunately, the results of these studies are difficult to interpret because the smears were reviewed by pathologists who knew the HPV DNA status of the spec-

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Table I. Population characteristics

Age (yr, mean ± SE)	17.1 ± 1.5
Age at initial sexual intercourse (mean ± SE)	15.5 ± 1.5
No. of lifetime sexual partners	
0-3	65%
4-10	25%
≥11	10%
No. of sexual partners in past 2 mo (mean ± SE)	1.2 ± 0.7
Race-ethnicity (%)	
White	71%
Black	5%
Hispanic	10%
Other	14%
Reason for visit (%)	
Family planning	90%
Sexually transmitted disease related	10%

imens at the time the reviews were conducted, possibly biasing the outcome. We describe a study that examines the association between HPV DNA status and diagnostic interpretations on review of cytologic results by two independent pathologists who were without knowledge of the HPV DNA results.

Methods

This study was part of a large epidemiologic study examining the prevalence of HPV DNA in sexually active adolescents.[10] All female adolescents (aged 13 to 19 years) who were attending a private, suburban family planning clinic and having a pelvic examination for any reason were asked to enter this study according to the guidelines set by the Committee for Human Research to screen for the presence of cervical HPV DNA. The refusal rate was <10%. Populations attending the clinic were predominantly middle-class and the racial-ethnic distribution of the clinic was 60% white, 20% black, 20% Hispanic, and 10% other. All female adolescents who entered the study were asked to complete a self-administered questionnaire before examination; this included demographic and sexual behavior information. Pelvic examination included a Papanicolaou smear obtained with a Dacron swab and spatula followed by an endocervical and exocervical sample for HPV DNA with a Dacron swab. Slides for cytologic testing were immediately fixed in alcohol and were sent to a contracted private cytology laboratory for initial review per routine protocol. Slides from 134 consecutive subjects during the second month through the fourth month of the study were identified and released for review by two independent pathologists. Although the two pathologists were without knowledge of the HPV DNA status of each specimen and were not apprised of the specific purpose of the review, they were asked to carefully review the slides for the epidemiology study.

Cytology. Papanicolaou smears were prepared by standard methods at the private cytology laboratory and were then screened according to the following routine protocol. All smears were first screened by a cytotechnician. Those smears found to have abnormal findings including inflammation, atypia, and changes suggestive of condyloma and neoplasia, as well as 10% of the benign smears, were then reviewed by a supervising pathologist. Cytologic diagnoses from the private laboratory were categorized into one of the following: benign, atypia, or cervical intraepithelial neoplasia with or without koilocytosis. The term *cervical intraepithelial neoplasia* is used for easier reference in this article although it is understood that cytologic testing cannot determine the site of origin of neoplastic cells.

Pathologist A then reviewed each slide independently without knowledge of either the HPV DNA status or the private laboratory's cytologic diagnosis at × 10 magnification with higher magnification used for the examination of questionable or abnormal areas. For comparison, the second pathologist (pathologist B) used a method more common to cytology laboratories; that is, a cytotechnician screened the smears first and then pathologist B verified the diagnosis of all the smears. The diagnoses reported by the reviewing pathologists were categorized as described here. Standard criteria were used to diagnose neoplasia and koilocytosis.[11] Atypia was diagnosed when rare abnormal cells (neoplastic or koilocytes) were found or if some but not all of the features of cervical intraepithelial neoplasia or koilocytosis were found on Papanicolaou smears. Since cytologic correlates for condyloma are controversial, cells suggestive of condyloma (koilocytes) but without the nuclear features of neoplasia also were categorized as atypia. Slides that appeared to have been air-dried or inadequately stained were excluded from the analysis. The presence or absence of endocervical cells was noted by the reviewing pathologists.

HPV DNA hybridization. HPV DNA was identified with a commercially available ribonucleic acid–DNA dot blot hybridization technique (ViraPap).[12] Cervical swabs for HPV were immediately placed into 1 ml of the sample transport medium and refrigerated at 4° C for no longer than 2 weeks before processing. Samples were processed with the ViraPap method according to the manufacturer's recommendation.[12] This method allows for identification of HPV types 6, 11, 16, 18, 31, 33, and 35. Unused portions of the samples that were positive for the HPV probe mixture were then studied under similar conditions to determine the HPV type more precisely. Samples were filtered onto three separate membranes, and each membrane was then hybridized with one of three separate radioactively labeled probe mixtures: type 6/11, type 16/18, or type 31/33/35.[13]

Table II. Comparison of cytologic diagnoses among pathologists A and B and the private cytologic laboratory for HPV-positive and HPV-negative subjects

	Privatate cytologic laboratory: HPV positive			*Privatate cytologic laboratory: HPV negative*		
	Benign	*Atypical*	*Cervical intraepithelial neoplasia*	*Benign*	*Atypical*	*Cervical intraepithelial neoplasia*
Pathologist A						
Benign	9	0	0	92	1	0
Atypia	5	1	0	6	0	0
Cervical intraepithelial neoplasia	4	5	0	0	2	0
Pathologist B						
Benign	13	0	0	93	1	0
Atypia	3	3	0	4	1	0
Cervical intraepithelial neoplasia	2	3	0	1	1	0

Data analysis. For each of the reviewers, χ^2 tests were performed to examine the relationship between HPV DNA status and diagnosis. Interrater reliability is reported for agreement between the two reviewing pathologists. The effect of rescreening was assessed by calculating *z* scores. This test examined whether rescreening of HPV-positive subjects and HPV-negative subjects resulted in a significant difference in the proportion of subjects with a diagnosis of cervical intraepithelial neoplasia by the private cytology laboratory (first screen), when compared with diagnoses assigned by pathologist A or B (rescreens). Because the private cytology laboratory did not diagnose any cases of cervical intraepithelial neoplasia, a second variable for cytologic diagnosis was created for comparison. This variable compared only two categories: benign and any abnormality (atypia or cervical intraepithelial neoplasia). The *z* test was also performed to examine the difference in the proportion of subjects with any abnormality.

Results

Population characteristics. Table I describes the demographic characteristics of the subjects including sexual behaviors and reasons for visit. Twenty-four subjects (19%) had positive HPV DNA results: 5 were positive for group 6/11, 11 for group 16/18, 4 for group 31/33/35, and 1 for both group 16/18 and group 31/33/35. Three subjects had undetermined types with the described dot blot method.

Interrater reliability between the pathologists. The overall interrater reliability between the two pathologists with three cytologic categories (benign, atypia, and cervical intraepithelial neoplasia with or without koilocytosis) was 79% ($\kappa = 0.28$, $p = 0.001$). When categories were subdivided into *benign* and *any cytologic abnormality,* the interrater reliability was 86% ($\kappa = 0.51$, $p = 0.001$). For the latter analysis, the interrater reliability was 75% ($\kappa = 0.51$, $p < 0.002$) in the HPV-positive group and 89% ($\kappa = 0.208$, $p < 0.03$) in the HPV-negative group.

Comparison of cytologic diagnosis and HPV DNA status. There was a significant association between abnormal cytologic findings and positive HPV DNA status for the private cytology laboratory, pathologist A, and pathologist B. The private cytology laboratory found that none of the patients had cervical intraepithelial neoplasia; however, 6 of 24 HPV-positive subjects had atypia, compared with 3 of 101 HPV-negative subjects ($\chi^2 = 10.98$, $p < 0.0009$). Pathologist A diagnosed abnormal cytologic findings in 15 of 24 women in the HPV-positive group (9 with cervical intraepithelial neoplasia and 6 with atypia) compared with 8 of 101 women in the HPV-negative group (2 with cervical intraepithelial neoplasia and 6 with atypia) ($\chi^2 = 42.2$, $p < 0.0001$). A similar association was found for pathologist B; 11 of 24 HPV-positive women had abnormal cytologic findings (5 with cervical intraepithelial neoplasia and 6 with atypia) and 7 of 101 HPV-negative women had abnormal cytologic findings (2 with cervical intraepithelial neoplasia and 5 with atypia) ($\chi^2 = 24.59$, $p < 0.0001$).

Comparison of cytologic findings among the private laboratory and pathologists A and B within HPV-positive and HPV-negative groups. Table II compares the cytologic diagnoses of the private cytology laboratory with those of pathologist A and pathologist B by HPV DNA status. Of the cervical intraepithelial neoplasia cases diagnosed, only 2 had grade 2; the rest had grade 1. Both cases of grade 2 were assigned the same diagnosis by both pathologist A and pathologist B; both of these women were HPV positive. Pathologist A diagnosed 38% of the HPV-positive patients as having cervical intraepithelial neoplasia, compared with none diagnosed by the private cytology laboratory ($z = 3.09$, $p < 0.005$); pathologist A diagnosed *any cytologic abnormality* in 63% of the HPV-positive patients, compared with 25% by the private cytology laboratory ($z = 2.39$, $p < 0.03$). In contrast, there was no significant difference in the proportion of abnormal cytologic smears diagnosed in the HPV-negative group. Specifically, 2% of HPV-negative subjects were diagnosed to have cervical intraepithelial neoplasia by pathologist A and none were diagnosed by the private cytology laboratory ($z = 0.714$, difference not significant). Similarly, no difference was found for diagnosing "any cytologic abnormality" in the HPV-negative group ($z = 0.15$; difference not significant). In addition, 5 of the 6 subjects who were HPV positive and were diagnosed as having atypia by the private cytology laboratory had cervical intraepithelial neoplasia. Two of the subjects with atypia and who were HPV negative had cervical intraepithelial neoplasia.

Pathologist B also found significantly more subjects with cervical intraepithelial neoplasia on rescreening the HPV-positive group than were diagnosed by the private cytology laboratory, 21% by pathologist B versus 0% by the private cytology laboratory ($z = 1.98$, $p < 0.05$). However, there was no difference between the private cytology laboratory and pathologist B in the proportion of subjects with a diagnosis of cervical intraepithelial neoplasia among the HPV-negative group ($z = 0.714$, difference not significant). Pathologist B diagnosed *any cytologic abnormality* in 46% of HPV-positive subjects, compared with 25% diagnosed by the private cytology laboratory ($z = 1.2$; difference not significant). There was also no significant difference found between the proportion of those subjects with a diagnosis of *any cytologic abnormality* between pathologist B and the private cytology laboratory among the HPV-negative group ($z = 0.15$, difference not significant).

When readings between the two groups were compared by each HPV category, the significant differences found between the two readings remained similar for each HPV group (data not shown). In addition, the presence or absence of endocervical cells did not influence the differences in diagnosis on reviews.

Comment

This study demonstrates that a significant proportion of HPV-positive women in our study were diagnosed as having cervical intraepithelial neoplasia when their Papanicolaou smears were rescreened. In contrast, cytologic rescreening from HPV-negative women did not result in a significant proportion of additional abnormal diagnoses. This association was less clear for diagnosing the category *any cytologic abnormality* because pathologist A was more likely than the private cytology laboratory to identify atypia on smears from HPV DNA–positive women, whereas no such association was found for pathologist B. However, the clinical implications for atypia are controversial and this cytologic category is considered less predictive of a significant lesion.

Our findings suggest that the reported insensitivity of the Papanicolaou smear is due in part to screening error. Currently, the Papanicolaou smear is the primary screening tool to identify women with cervical disease, specifically intraepithelial neoplasia. Although this kind of cytologic screening remains the most cost-effective method, numerous studies have identified the limitations of the Papanicolaou smear, including substantially lower sensitivity rates than those of colposcopy and histology.[4-6] These limitations have been attributed to sampling error, difficulty in cytologic interpretation, and fatigue (in both cytotechnicians and pathologists) resulting in screening error.[14, 15] Although most laboratories set high standards for quality assurance, previous studies have documented that reviews of cytologic smears will result in a fivefold increase in the number of significant abnormalities as compared with the results from the initial routine screening.[16] Our study both confirms and extends these findings. We showed that additional abnormalities were found when cytologic smears were reviewed. However, when the rescreening results were analyzed by HPV status, the majority of additional significant abnormalities were present in the HPV DNA–positive group even though the reviewers were blinded to the HPV DNA status. This finding suggests that it would be more efficacious to review smears from this positive population.

Although our findings apppear to have important implications for the cytologic identification of cervical intraepithelial neoplasia, these results must be interpreted with caution. First, the clinical significance of the reviews is unknown because we did not perform colposcopic examinations and obtain histologic confirmations. It is possible that some of our reviews reflect erroneous diagnoses; however, this is unlikely since the positive predictive value of the Papanicolaou smear to identify disease is considered to be relatively high.[6] Our pathologists used strict criteria to diagnose disease. In addition, statistical analysis of our data indicated that the association between HPV DNA status and abnormal reviews was greater than chance alone.

Second, our findings may not necessarily be generalizable because the quality of screening can vary considerably among laboratories.[16] As a result, it is possible that our findings simply reflected a higher-quality screening process. However, we feel that our results were not biased by this possibility for several reasons. First, it should be stressed that, although the reviewers were without knowledge of the HPV status, they were aware that they were reviewing slides for a study that possibly reflected an increased "effort per slide" and were reviewing slides without the conventional pressures associated with rapid bulk screening. This situation may reflect a similar situation found when slides are reviewed for routine quality assurance measures. In addition, if the reviewers were significantly "better" at screening, it would have been more likely that additional abnormalities would have been found proportionately in both groups. Finally, the percent agreement between the pathologists reflected "differences in opinion" similar to those found in other studies.[17] To help remove the "type-of-reader" bias, we selected two types of review: One involved an independent pathologist and the other involved a screening cyto-technician-pathologist team. The two groups occasionally disagreed; however, both were significantly more likely to find additional abnormalities in the HPV-positive group and not in the HPV-negative group.

Another factor that influences the generalizability of our findings is the population examined. Our population consisted of sexually active young women with a high prevalence of HPV and cervical intraepithelial neoplasia (14% had cervical intraepithelial neoplasia on the reviewed smears). The prevalence of these factors may affect the outcome in such studies.

Last, our abnormal smears with cervical intraepithelial neoplasia were associated with a high rate of DNA positivity (86%). This rate is higher than that reported in other studies,[18, 19] which may reflect the high prevalence of these specific HPV types in our population and the association between these types and abnormal cytologic findings.

In summary, reviewing cytologic results in patients who are HPV DNA positive increased the number of diagnosed cases of cervical intraepithelial neoplasia. This suggests that in a population at high risk for HPV and cervical intraepithelial neoplasia but with normal cytologic findings, routine screening for HPV DNA would be beneficial if the cytologic findings from HPV DNA–positive women are reviewed. Longitudinal studies that include colposcopy and histology are currently under way to confirm these findings.

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The effect of trisomy 18 on transverse cerebellar diameter

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The transverse cerebellar diameter was measured in 19 fetuses with trisomy 18. Eleven of the cerebellar measurements (57.9%) were >2 SD below the mean for the patient's gestational age. If fetal biometry rather than last menstrual period were used to determine gestational age, four of the 19 (21.0%) cerebellar measurements were >2 SD below the mean. Both intrauterine growth retardation and intrinsic central nervous system abnormalities associated with trisomy 18 appeared to affect cerebellar size. (Am J Obstet Gynecol 1991;165:72-5.)

Key words: Transverse cerebellar diameter, trisomy 18

The ultrasonographic findings associated with trisomy 18 have been well documented.[1, 2] The clinical features of this syndrome are numerous and vary from case to case.[3] Although malformations of the central nervous system are relatively uncommon, cerebellar abnormalities have occasionally been reported.[4] Inagaki et al.,[5] for example, found a small cerebellum (and hydrocephalus) in five newborns with trisomy 18. To date there have been only a few reports on the antenatal evaluation of the cerebellum and cisterna magna in fetuses with trisomy 18. Comstock and Boal[6] reported two cases and Thurmond et al.[7] five cases of third trimester trisomy 18 fetuses with a small cerebellum and an enlarged cisterna magna. The purpose of our investigation was to determine if the transverse cerebellar diameter was consistently smaller than expected in a larger series of second-trimester, as well as third-trimester, trisomy 18 fetuses.

Material and methods

We retrospectively ascertained all consecutive karyotypic diagnoses of trisomy 18 made at Magee-Womens Hospital from May 1986 to May 1990 and identified those women who had an ultrasonographic examination. Exclusion criteria included insufficient dating parameters (see below) or intrauterine fetal death at the time of the ultrasonographic examination.

For entry in the study these criteria for ascertaining dates were required: regular menses, no oral contraceptives for 3 months before conception, an early pregnancy test, and a first pelvic examination at or before 10 weeks' gestation.

Twenty-one fetuses with a karyotypic diagnosis of trisomy 18 met these entrance criteria. In two cases an Arnold-Chiari type II malformation prohibited visualization of the cerebellum. The latter cases were excluded from further analysis; hence there were 19 fetuses in the study group.

A thorough ultrasonographic examination was performed with a linear array real-time system with a 3.5 or 5.0 MHz transducer (General Electric RT3000, RT3200, General Electric Corp., Milwaukee). A composite assessment of gestational age was obtained from standard biometric measurements of biparietal diameter, femur length, head circumference, and abdominal circumference. The amount of amniotic fluid and the placental site were documented.

We have consistently measured the transverse cerebellar diameter as part of our routine anatomic survey since 1986. A slightly posterior angulation from the plane of the biparietal diameter allows visualization of the cerebellum (Fig. 1). The normal cerebellar diameter for our population as a function of gestational age was derived prospectively from 675 patients with normal pregnancy between 14 and 42 weeks' gestation according to above-noted dating criteria.[8] A transverse cerebellar diameter ≥2 SD below the mean for gestational age was defined as abnormal (Fig. 2).

Results

The transverse cerebellar diameters in the 19 fetuses with trisomy 18 are plotted on Fig. 3 versus gestational age by a reliable last menstrual period. Eleven of the cerebellar measurements (57.9%) were ≥2 SD below the mean. When the transverse cerebellar diameter was compared to the patient's gestational age by fetal biometry rather than by last menstrual period, only four of

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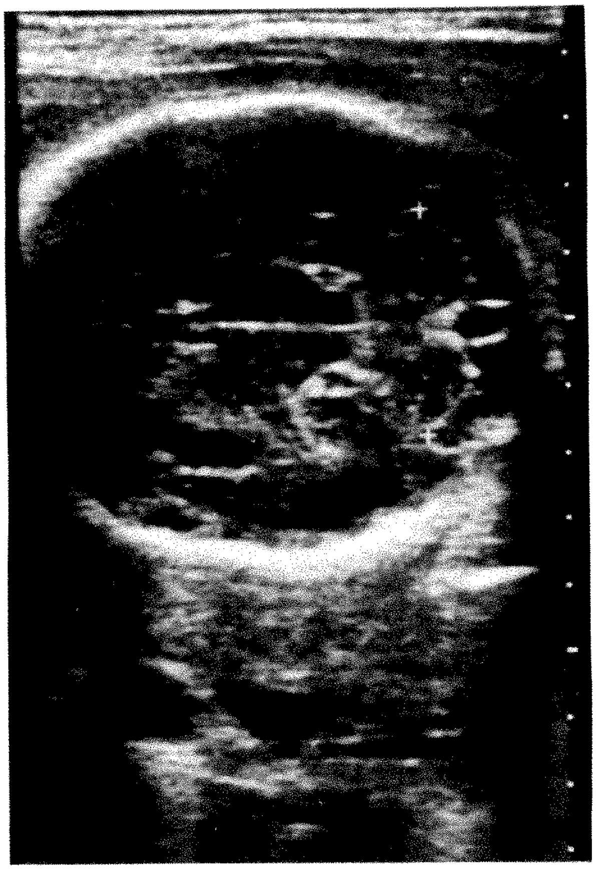

Fig. 1. *Graticules* demarcate transverse cerebellar diameter in chromosomally normal fetus at 29 weeks' gestation.

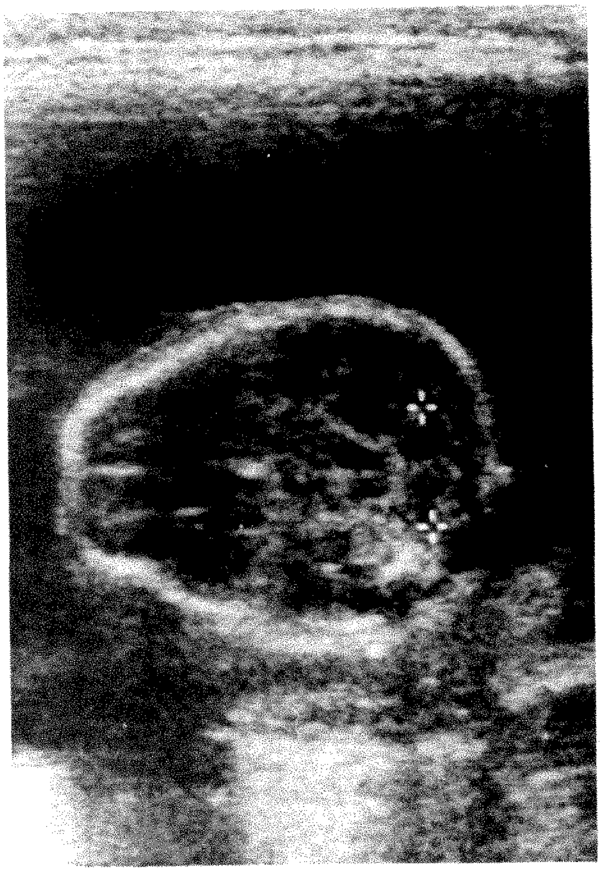

Fig. 2. Trisomy 18 fetus with cerebellar hypoplasia at 31 weeks' gestation. *Graticules* demarcate transverse cerebellar diameter of 22 mm.

19 measurements (21%) were >2 SD below the mean. Consequently, if a patient's dates are uncertain, the transverse cerebellar diameter, as well as standard biometry, may give rise to an incorrect estimation of gestational age.

The likelihood that the transverse cerebellar diameter would be small appears to increase with gestational age—two of seven fetuses (28.6%) before 21 weeks' gestation versus nine of 12 (75%) third-trimester fetuses had a cerebellar diameter >2 SD below the mean.

In contrast to the findings of Comstock and Boal[6] and Thurmond et al.,[7] the cisterna magna was enlarged (≥1.1 cm)[9] in only three of the 12 fetuses evaluated in the third trimester.

Four of the 19 fetuses had normal fetal biometry at the time of their ultrasonographic examination (17, 17, 19.4, and 30.8 weeks, respectively); two of the fetuses in this group had a normal transverse cerebellar diameter, and two had cerebellar hypoplasia. Of the 15 growth-retarded trisomy 18 fetuses, six had a normal transverse cerebellar diameter and nine had a transverse cerebellar diameter >2 SD below the mean. Hence there appears to be two potential mechanisms for the presence of cerebellar hypoplasia in fetuses with trisomy 18: an inherent abnormality in brain growth or a generalized inhibition of fetal growth.

Comment

Edwards' syndrome (trisomy 18)[8] was first described in 1960. The frequency of trisomy 18 is between 1 in 3500 and 1 in 8000 births.[10] However, these figures do not take into account the spontaneous in utero attrition rate. Between 16 weeks' gestation and term 68% of trisomy 18 fetuses either will abort or will be stillborn.[11] The ultrasonographer may therefore encounter fetuses with trisomy 18 more than twice as frequently as the birth statistics indicate.

The neuropathologic changes associated with trisomy 18 tend to be quite variable and hence are not diagnostic. Hydrocephalus with or without meningomyelocele, agenesis of the corpus callosum, and a small cerebellum are among the central nervous system malformations that are potentially detectable with antenatal ultrasonography.[12] Even when the cerebellum appears to be normally formed, a microscopic examination may reveal numerous structural alterations.

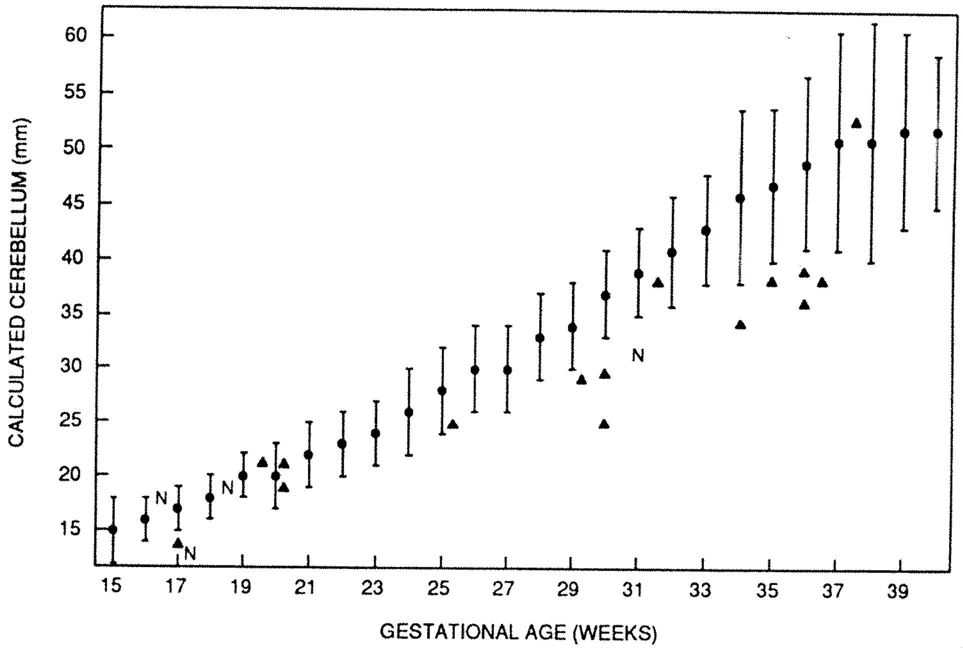

Fig. 3. Calculated mean (± 2 SD) for transverse cerebellar diameter versus gestational age in weeks. Trisomy 18 fetuses: *N*, Adequate growth; ▲, growth retardation.

The association between chromosomal abnormalities and intrauterine growth retardation is well known.[13] The severity of growth delay with trisomy 18 appears to be variable in time of onset and in severity. Although birth weights for fetuses with trisomy 18 are generally reduced, the range is quite wide.[14]

Growth of the fetal cerebellum begins later, finishes sooner, and occurs faster than does the growth of other parts of the brain. The vulnerability of the brain during its growth spurt has been well documented.[15] In a previous study[16] of 44 chromosomally normal, small-for-gestational-age fetuses, the transverse cerebellar diameter was >2 SDs below the mean in 26 (59.1%). There was a wide variation in transverse cerebellar diameter measurements at each weight percentile, emphasizing the importance of the severity, duration, and type of growth inhibition. Our current results indicate that cerebellar size in fetuses with trisomy 18 may be affected not only by an inherent inhibition of brain growth but also by the generalized disturbance of overall size associated with this specific chromosomal abnormality.[17] However, the presence of significant growth retardation did not result in cerebellar hypoplasia in every case; two fetuses in the third trimester (31.7 and 38 weeks' gestation) and three second-trimester fetuses affected by early growth retardation (20, 20, and 23 weeks' gestation) had a normal transverse cerebellar diameter.

Although other authors[6, 7] have reported an association between trisomy 18 and an enlarged cisterna magna, nine of 12 third-trimester fetuses in the current investigation had a normal cisterna magna. Nakamura et al.[18] have reported hypoplasia of the bony posterior cranial fossa in three of nine trisomy 18 neonates, resulting in a small cisterna magna or its absence. Hence the difference in results between the current and previous[6, 7] studies may be due to: (1) structural differences within the posterior fossa between trisomy 18 fetuses, (2) the severity of growth retardation, or (3) sample size.

Our findings with respect to the transverse cerebellar diameter and cisterna magna once again confirm the wide scatter about the mean for almost any biometric measure that has been obtained in trisomy 18 fetuses.[19]

The ultrasonographic findings associated with trisomy 18 vary considerably. Benacerraf et al.[2] were able to detect 12 of 15 (80%) trisomy 18 fetuses by using the ultrasonographic criteria of abnormalities of the face and limbs or a diaphragmatic hernia. The ultrasonographic detection of cerebellar hypoplasia should alert the ultrasonographer to the possibility of trisomy 18. However, measurement of the transverse cerebellar diameter or evaluation of the cisterna magna cannot replace a thorough anatomic survey in the detection of this particular chormosomal abnormality.

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Porencephalic cyst: A complication of fetal intravascular transfusion

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A case of unilateral porencephalic cyst as a result of intravascular intrauterine transfusion is reported. Although other factors may have contributed, fetal bradycardia and relative hyperviscosity as a result of increased fetal hematocrit value were considered to be related to this event. Because some authors recommend direct intravascular transfusion to supraphysiologic hematocrits to prolong the interval between procedures, we would caution the use of this approach on the basis of theoretic considerations and the occurrence of this complication. (AM J OBSTET GYNECOL 1991;165:76-8.)

Key words: Porencephalic cyst, intravascular transfusion, intrauterine transfusion, Rh alloimmunization

Direct fetal intravascular transfusion has become an important method of treating the anemic fetus. Reported complications of intravascular transfusion include umbilical cord hematoma and fetal bradycardia. We report a patient treated for Rh alloimmunization whose fetus developed a left cerebral porencephalic cyst after intravascular transfusion complicated by fetal bradycardia and an elevated fetal hematocrit value.

Case report

A 28-year-old gravida 2, para 1 woman presented at 12 weeks' gestation for prenatal care with an anti-D titer of 64. In her only previous pregnancy she was delivered by cesarean section at term because of fetal distress of a 3992 gm male infant whose blood type was reportedly Rh negative. The patient gave an otherwise negative medical history with the exception of having received a blood transfusion at age 16 years for undetermined reasons. Her husband's blood type was A positive, heterozygous for the D antigen.

At 21.4 weeks' gestation, the indirect Coombs titer rose to 512 and fetal blood sampling was performed through an anterior fundal placenta after documentation of normal results of an ultrasonographic examination. The fetal hematocrit was 34.9% and the fetal blood type was O positive. An indirect Coombs titer of 8192 obtained at 24.1 weeks' gestation to exclude an amnestic maternal antibody response necessitated repeat fetal blood sampling. The hematocrit value was 28.8%.

Five days later, fetal ascites and a small pericardial effusion were noted on ultrasonographic examination at the time of preparation for intrauterine transfusion. Pretransfusion fetal hematocrit value was 17%. Forty milliliters of filtered maternal packed red blood cells (hematocrit, 84%) were transfused intravascularly into the fetus. Blood was filtered twice, once through a leukocyte-poor filter during blood bank processing and again with a standard blood transfusion filter at the time of transfusion. The interval fetal hematocrit value was 55%. Umbilical venous pressure was noted to rise from 10 mm Hg at the onset of the procedure to 23 mm Hg after completion of the transfusion. Nine milliliters of fetal blood was slowly removed to reduce umbilical venous pressure and avoid hyperviscosity. At the completion of the procedure, a fetal bradycardia to 60 beats/min was noted, resolving spontaneously after 7 to 10 minutes.

Ultrasonographic examination at 26.1 weeks' gestation, before the second intrauterine transfusion, demonstrated a new finding of left cerebral ventricular enlargement (Fig. 1). Transfusions were repeated at 26.1, 28.1, 31.1, and 33.9 weeks' gestation without difficulty. There was no progression of the cystic cerebral lesion on serial ultrasonography. A 2200 gm female infant with Apgar scores of 7 at 1 minute and 9 at 5 minutes was delivered by repeat cesarean section at 35.9 weeks' gestation. Phototherapy was required for 3 days. Computed tomography scan without contrast on the day 9 after birth demonstrated atrophic changes of the left cerebral hemisphere with atrophic enlargement of the left lateral ventricle (Fig. 2). The infant was discharged home on the day 10 after birth; neurologic status was noted to be normal.

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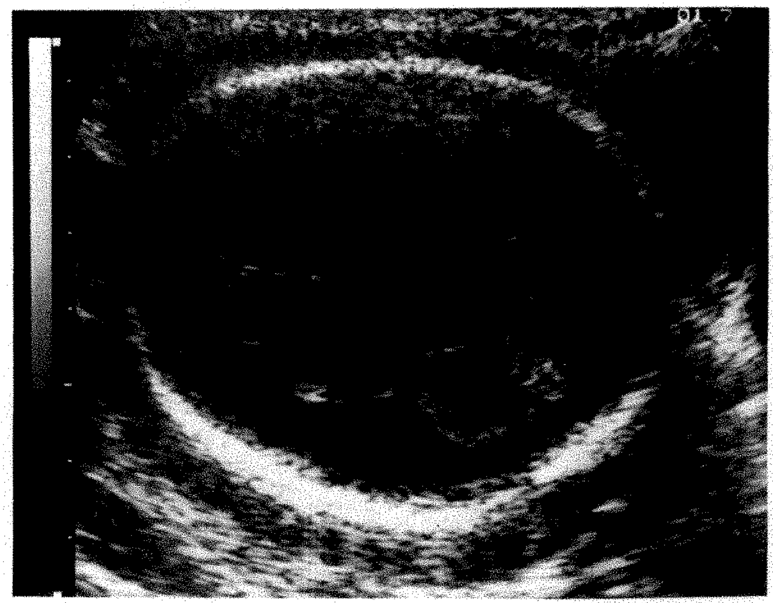

Fig. 1. Ultrasonographic examination of fetal head at 26.1 weeks' gestation, 9 days after the initial intrauterine transfusion. A cystic structure was noted in the left cerebral hemisphere.

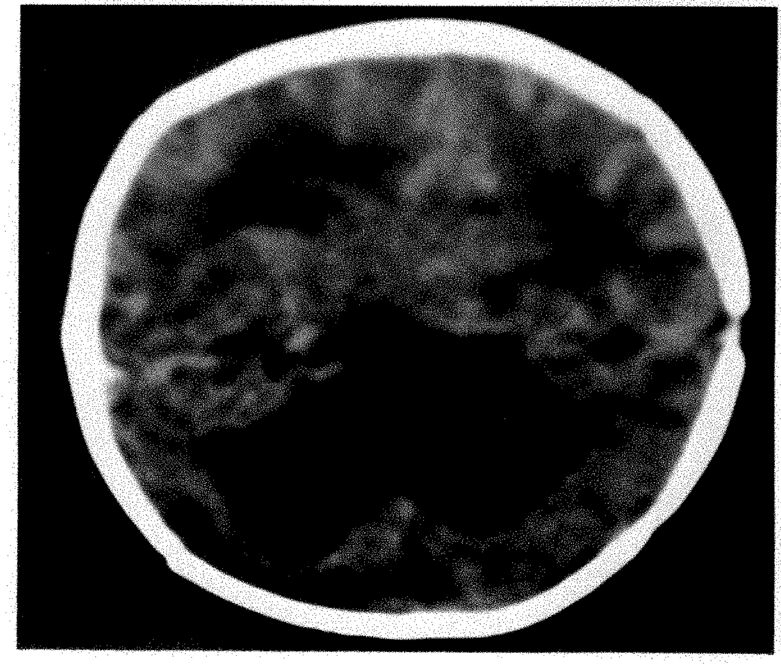

Fig. 2. Computed tomography of the head without contrast on day 9 after birth showing a left-sided porencephalic cyst with cerebral atrophy.

Comment

To our knowledge, congenital malformations as a result of intrauterine transfusion have not been previously described. The potential exists for vascular accidents to occur as a result of several mechanisms. Air embolism may potentially occur during intravascular transfusion; however, at our institution a closed circuit transfusion system with a three-way stopcock is used, and the apparatus is flushed with blood at each step of the procedure to avoid air leakage. Another potential explanation of vascular accidents might be that of microemboli from transfused blood; however, we minimize this risk by filtering the packed red blood cells twice before transfusion. Alterations in regional blood flow during these procedures may theoretically occur. Two mechanisms that are potentially contributory to the development of the porencephalic cyst in our case include the dramatic increase in blood viscosity and concommitant fetal bradycardia. In the adult, blood hyperviscosity is a recognized cause of cerebral ischemia and infarction.[1] In addition, fetal bradycardia encountered during the intravascular transfusion in our case may have contributed to the cerebrovascular event by producing hypotension, sluggish blood flow, and markedly reduced cerebral perfusion.

Controversy exists with regard to the optimal method of transfusion for hemolytic disease of the fetus. Some centers use only the intravascular method, routinely transfusing packed red blood cells to target hematocrit values of at least 55%.[2] We routinely use the intravascular technique, achieving hematocrit values in the range of 35% to 40%, followed by a standard intraperitoneal transfusion to lengthen the interval between procedures.

Until more data concerning the effects of relative hyperviscosity on fetal organ perfusion become available, we recommend intravascular transfusion of blood to physiologic hematocrits. A combined technique of intrauterine transfusion that includes the intraperitoneal transfusion may be used effectively to allow prolonged intervals between successive transfusions.

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Hyporegenerative anemia associated with intrauterine transfusion in rhesus hemolytic disease

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Serial erythropoietin and ferritin levels were monitored in a fetus and newborn requiring three intravascular transfusions (in utero) for severe Rh disease. The newborn had a hematocrit of 37% at birth; however a hyporegenerative transfusion-dependent anemia developed and lasted approximately 3 months. The prolonged hyporegenerative anemia may be caused in part by erythropoietin suppression as a result of the fetal intravascular transfusions. In addition, anti-D antibody may also contribute to this anemia by a direct toxic effect on erythroid precursors and by peripheral hemolysis of reticulocytes. (AM J OBSTET GYNECOL 1991;165:79-81.)

Key words: Intravascular transfusion, erythropoietin, hyporegenerative anemia, Rh disease

Severe hemolytic disease of the fetus has declined in frequency over the past two decades because of successful prevention with Rh immune globulin. Unfortunately, this disease continues to be seen with regularity. Intravascular fetal transfusion in lieu of intraperitoneal transfusion has become the technique of choice for treating severe hemolytic disease of the fetus.[1] Hyporegenerative anemia of the newborn after intravascular transfusion for treatment of severe hemolytic disease of the fetus is seen frequently at our institution. Observed after fetal intravascular transfusion, this anemia is delayed in onset until the second to fourth week of life and is associated with low reticulocyte counts. The pathogenesis of this hyporegenerative anemia is unknown; however, both immune injury to the bone marrow[2] and erythropoietin suppression[3] are implicated. We therefore studied serial erythropoietin levels and antibody titers in a newborn who underwent three intravascular transfusions in utero and remained transfusion dependent for 12 weeks after birth.

Material and methods

Informed consent was obtained before samples were taken from the fetus or newborn. Percutaneous umbilical blood sampling and fetal intravascular transfusions were performed under ultrasonographic guidance using standard techniques. Serum erythropoietin and ferritin levels were performed by radioimmunoassay (Smith-Kline Laboratories, Van Nuys, Calif.), and the hemoglobin, hematocrit, reticulocyte counts, and anti-D antibody titers (indirect antiglobulin test) were measured with standard techniques. All samples for erythropoietin, ferritin, and anti-D titers were frozen at −70° F and assayed simultaneously.

Case report

A 27-year-old white woman, gravida 2, para 1, was noted to have a maternal serum anti-D titer of 64 and an anti-C titer of 32 at her first prenatal visit; therefore she had follow-up with serial amniocentesis. The cause of her sensitization was uncertain, because she received both antepartum and postpartum Rh immune globulin in her first pregnancy and had never received blood products. At 28 weeks' gestation, the anti-D titer was 128, and the optical density at 450 nm rose sharply into zone III of the Liley curve. Therefore a percutaneous umbilical blood sampling procedure and possible fetal transfusion were planned. A percutaneous umbilical blood sampling procedure was performed through an anterior placenta and demonstrated the following results on fetal blood: hematocrit, 26%; erythropoietin level, 4 mU/ml; fetal blood type, O Rh positive; and direct antiglobulin test, positive. Unfortunately, reticulocyte counts were not obtained on fetal blood. An intravascular transfusion of 36 ml of type O Rh-negative, cytomegalovirus-negative, irradiated, packed erythrocytes was performed and resulted in a final hematocrit of 36%.

By 30 weeks' gestation the anti-D titer rose to 16,384, and ascites had developed in the fetus. Presumably the significant rise in the anti-D antibody titer was the result of an anamnestic immune response, which was caused by a fetomaternal hemorrhage from the initial transfusion. A repeat percutaneous umbilical blood sam-

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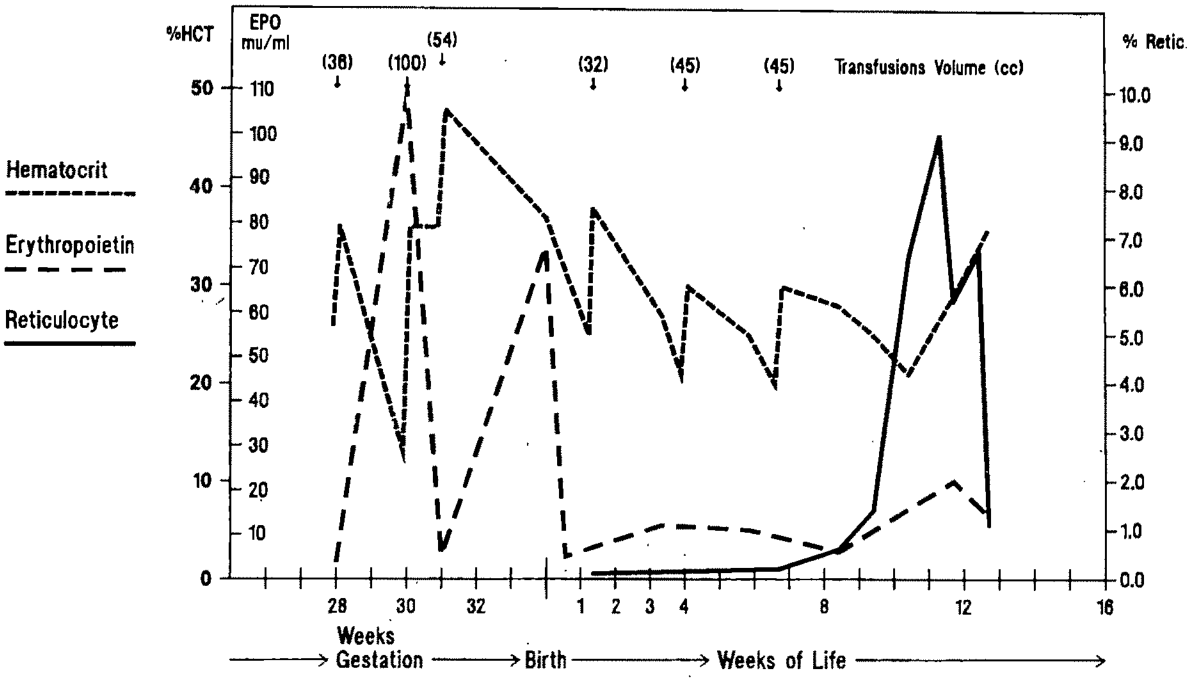

Fig. 1. Severe transfusion-dependent hyporegenerative anemia after intravascular transfusion for rhesus hemolytic disease of fetus. Erythropoietin levels *(EPO)*, milliunits per milliliter, hematocrit (percent), and reticulocytes (percent) are depicted in fetus from 28 weeks until birth and in newborn from birth to 12 weeks. *Arrows* (top of graph) depict transfusion volumes in fetal and newborn periods. Severe Rh disease in this pregnancy necessitated three intravascular transfusions and early delivery at 34 weeks' gestation. At birth cord blood consisted of 100% type O, Rh-negative adult donor blood, with absence of fetal blood cells by the Kleihauer-Betke method. Severe transfusion-dependent hyporegenerative anemia ensued with inappropriately low reticulocyte counts and erythropoietin levels. Three neonatal transfusions were necessary until erythropoietin levels and reticulocyte counts increased by 12 weeks after birth.

pling procedure revealed a fetal hematocrit of 13% and an erythropoietin level of 109 mU/ml. An intravascular transfusion with 100 ml of similarly prepared donor blood resulted in a hematocrit of 36%. After the transfusion, the percentage of fetal cells in the fetal blood sample was 0.5% by the Kleihauer-Betke method.

A third percutaneous umbilical blood sampling procedure was performed at 32 weeks' gestation, revealing a fetal hematocrit of 36% and an erythropoietin level of 5 mU/ml. An intravascular transfusion of 54 ml of similarly prepared donor blood resulted in a hematocrit of 48%.

At 34 weeks' gestation the fetus was in a breech presentation, the amniotic fluid volume was diminished, and a mature phospholipid profile was present. Cesarean section resulted in a vigorous and healthy appearing male infant weighing 2240 gm and having no physical manifestations of rhesus hemolytic disease. The cord blood was type O, Rh negative, and fetal cells were absent by the Kleihauer-Betke test. The erythropoietin level on the cord blood was 75 mU/ml and the initial hematocrit of the infant was 37%. The infant appeared healthy, except for hyperbilirubinemia that necessitated phototherapy and reached a peak of 11 mg/dl on the third day of life.

On day 4 of life, the erythropoietin level had dropped to 5 mU/ml, and by the ninth day of life the infant had tachycardia, poor feeding, and pallor. At that time the hematocrit was 25% with a reticulocyte count of 0.1%; he was given a simple transfusion of 32 ml (approximately 15 mg/kg) of type O, Rh-negative blood, which resulted in resolution of his symptoms. During the next 2 months anemia recurred and was marked by low reticulocyte counts and symptoms of cardiovascular compromise, necessitating two additional transfusions (Fig. 1). By 3 months of age the erythropoietin level rose to 22 mU/ml, and the hematocrit and reticulocyte levels recovered. He was feeding and growing normally.

The fetal and newborn serum anti-D titers (indirect globulin tests) were as follows: 16 at 28 weeks' gestation, 32 at 30 weeks' gestation, 128 at 31 weeks' gestation, 1024 at birth, 512 at 3 days of life, 128 at 3 weeks of life, 1 at 6 weeks of life, and 1 at 14 weeks of life. Serum ferritin levels were measured periodically and gradually increased from 170 ng/ml at 28 weeks' gestation to 672 ng/ml at birth. At 3 weeks of life the ferritin rose to 984 ng/ml.

Comment

Delayed anemia in infants with Rh hemolytic disease is well described in the literature[1-6] before 1963 when intrauterine (intraperitoneal) transfusions were first reported. Dillon and Krivit[4] performed serial bone marrow examinations in 14 newborns with Rh hemolytic disease, and in only one case did the normoblast counts fall below the normal range, suggesting a hypoplastic marrow. In this one case[4] the infant had received three exchange transfusions in the early newborn period. The majority of their cases[4] (13/14) were associated with normoblastic hyperactivity in the bone marrow. These authors and other[5, 7] have suggested that this delayed anemia may be related to many factors, including peripheral hemolysis of fetal Rh-positive cells, a reduced survival of donor Rh-negative cells, and rapid body growth.

Burk et al.[8] described a newborn with severe Rh disease (requiring three intrauterine transfusions) who experienced transfusion-dependent anemia for 12 weeks after birth. These authors did not specify whether the intrauterine transfusions were performed by the intraperitoneal or the intravascular route. At 7 weeks after birth the baby had severe anemia (hemoglobin, 4.5 gm/dl) and a low reticulocyte count (0.1%). Therefore a bone marrow aspiration was performed. This demonstrated many normoblasts; however, there were no reticulocytes. An eluate prepared from the bone marrow erythrocyte precursor cells contained anti-D antibody. These authors[8] concluded not only that the antibody was of sufficient titer to support a hemolytic process but also that it affected bone marrow precursors, leading to an apparent failure of reticulocytosis. The infant had an Rh-negative blood type with a negative direct but positive indirect Coombs' test (anti-D titer was 128). This suggested that only adult Rh-negative cells from previous transfusions survived in the infant's peripheral circulation.

Koenig et al.[3] reported decreased serum erythropoietin levels in an infant with Rh hemolytic disease and hyporegenerative anemia. The fetus had received two intravascular transfusions at 26 and 31 weeks' gestation and was delivered with hydrops at 32 weeks' gestation. At 2 weeks of life the newborn had a hematocrit of 29% and absence of reticulocytes; by 4 weeks of life a hematocrit of 17% prompted a transfusion. These investigators also speculated that reduced erythropoietin concentrations contributed to the anemia in this newborn. Our data also are consistent with those of Koenig et al.,[3] demonstrating an inappropriately low erythropoietin level in the presence of severe newborn anemia. One would expect a very high erythropoietin level if the anti-D antibody were the only cause of this prolonged anemia. Thus there appear to be at least three mechanisms involved in this anemia: anti-D destruction of bone marrow precursors, anti-D destruction of reticulocytes in the peripheral circulation, and an inappropriately low erythropoietin level.

As with other anemias in newborns, iron deficiency does not appear to be a cause of hyporegenerative anemia, as evidenced by the high ferritin levels in this case. Fetal serum ferritin levels appear to rise dramatically in response to intrauterine transfusion. Likewise, the newborn with hyporegenerative anemia also is observed to have high ferritin levels. This reflects both an increase in total iron load as a result of transfusions and a reduced ferritin use as a secondary consequence of erythropoietin suppression.

The findings in this case and in previous reports support future study of recombinant erythropoietin, alone or in combination with exchange transfusion, as a treatment for prolonged hyporegenerative anemia after multiple intrauterine transfusions.

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Daily administration of the progesterone antagonist RU 486 prevents implantation in the cycling guinea pig

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Since progesterone is required to prepare the endometrium for implantation of an embryo, a progesterone antagonist may inhibit nidation and thus prevent pregnancy. We addressed this possibility in the guinea pig, the small laboratory animal whose reproductive physiology most resembles that of women. Daily administration of the antiprogestin RU 486 (0, 1, 2, or 3 mg/kg, subcutaneously) for 9 days after mating inhibited implantation in a dose-dependent fashion. When this compound was given daily throughout the estrous cycle, cyclic vaginal changes, ovulation, and mating were suppressed in up to 17%, 28%, and 55% of animals, respectively. Two of seven mated female animals receiving RU 486, 1 mg/kg/day, had implantation sites. Nidation was completely blocked at higher doses. Thus daily antiprogestin administration prevented pregnancy in sexually active, normally cycling guinea pigs. A similar strategy using a daily antinidatory dose of an antiprogestin may offer a novel approach to human fertility control. (AM J OBSTET GYNECOL 1991;165:82-6.)

Key words: RU 486, guinea pig, contraception, implantation

There is a great worldwide need for the development of new contraceptive agents for population control. One attractive strategy is to interdict implantation without suppressing ovulation or steroidogenesis. Since progesterone is required for the differentiation of the endometrium in preparation for pregnancy,[1] we hypothesized that interruption of the action of progesterone might create a hostile endometrium that is incapable of accepting an embryo. Synthetic steroids that competitively antagonize progesterone at its receptor may thus offer a novel approach to fertility control.

To address this possibility, we investigated the effects of continuous daily administration of RU 486, a progesterone antagonist with very little progestational activity,[2] on the reproductive function of the guinea pig. Since our goal was to develop a human contraceptive agent, we chose to study an animal whose reproductive physiology was similar to that of women. Among the small laboratory animals, the guinea pig best fits this criterion. The guinea pig ovulates spontaneously and has a 16-day estrous cycle, a prolonged luteal phase as a result of an actively secreting corpus luteum, and a hemochorial placentation that is dependent on progesterone.[3, 4] Its reproductive cycle can be timed conveniently by examination of the vaginal membrane, which opens at the time of estrus and ovulation.[5]

Material and methods

Guinea pigs. This study was approved by the Animal Care and Use Committee of the National Institute of Child Health and Human Development. Male and female Hartley guinea pigs (Charles River Breeders, Wilmington, Mass.) weighed 900 to 1200 and 550 to 950 gm, respectively, at the beginning of the studies. They were kept at room temperature (20° to 22° C) under controlled light conditions and had free access to pelleted food and tap water, according to National Institutes of Health guidelines.

Preparation of RU 486. RU 486 was obtained as micronized powder from Roussel-Uclaf (Paris). It was injected subcutaneously daily at 8 to 10 AM in 0.1 to 0.2 ml of a benzyl benzoate and castor oil (1:2.5, vol/vol) solution. Control animals received oil vehicle only.

Procedures. The aspect of the vaginal membrane was examined daily at 8 to 10 AM. When the membrane was fully open, vaginal smears were taken by irrigating the vagina with a few drops of saline solution. The unstained cells were examined under a microscope and

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Table I. Effects of RU 486 on implantation rate and corpus luteum function in guinea pigs treated for 9 days after mating

Dose (mg/kg/day)	*No.*	*Implantation sites*	*Corpora lutea*	*Progesterone* (ng/ml)*	
				Day 7	*Day 10*
0	10	35† (8‡)	42† (10‡)	1.6 ± 0.1	1.8 ± 0.2
1	10	32 (8)	36 (9)	1.4 ± 0.2	1.4 ± 0.2
2	10	13 (4)	39 (9)	1.3 ± 0.2	1.3 ± 0.2
3	10	0 (0)	41 (10)	1.9 ± 0.3	1.1 ± 0.1§

*Mean ± SE on postcoital day 7 or 10.

†Total number of implantation sites or corpora lutea.

‡Number of animals with implantation sites or corpora lutea.

§Significantly different, compared with control group ($p = 0.004$).

categorized according to the description of Young.[5] The day of leukocytic infiltration after a cornified smear, designated cycle day 1, normally follows ovulation within 24 hours. Blood drawing and intravascular injections were performed by puncture of the anterior vena cava or right ventricle with the animal under anesthesia with ketamine hydrochloride (25 mg/kg intraperitoneally) and xylazine (5 mg/kg intraperitoneally). Plasma was stored at −20° C for later progesterone measurements. Fifteen minutes before the animal was put to death, 1 ml of Evans blue dye 1% was injected intravascularly to facilitate the detection of implantation sites.[6] The animals were killed with carbon dioxide. The number of implantation sites and corpora lutea were determined by visual inspection of the uteri or by examination of the ovaries under a dissecting microscope.

Study 1: Effects of postcoital administration of RU 486. The first study evaluated the ability of postcoital administration of RU 486 to prevent implantation. All animals were acclimatized initially to a 14-hour-light/10-hour-dark cycle (lights on at 7 AM). Female animals were chosen for the study if they had a previous 13- to 17-day cycle, as determined by two subsequent openings of the vaginal membrane. They were individually caged with a male 4 to 5 days before the next expected opening of the vaginal membrane and checked daily at 8 to 10 AM for signs of mating. The day after copulation, designated postcoital day 1, was documented by the presence of a copulatory plug or by the presence of spermatozoa in the vaginal smear. RU 486 was given in single daily doses of 0, 1, 2, or 3 mg/kg for the first 9 days after mating to groups of 10 female animals. Progesterone was measured on postcoital days 7 and 10 and necropsy was performed on day 10.

Study 2: Effects of precoital administration of RU 486. The second study extended RU 486 treatment to the entire cycle before mating and evaluated its effects on sexual behavior, ovarian activity, and implantation rate. All animals were acclimatized initially to an inverted 14-hour-light/10-hour-dark cycle (lights on at 8 PM). Females with a previous 15- to 17-day estrous cycle were randomized to four groups of 12 animals. RU 486 injections (0, 1, 2, or 3 mg/kg/day, subcutaneously) were begun on cycle day 2 and continued until 1 day before the animal was put to death. Progesterone was measured on cycle day 8. Two days later, each female was caged with a male of proved fertility. An intensive search for copulatory evidence was then undertaken, because our previous study had shown that these signs were often missed if animals were checked only once daily. Accordingly, female animals were examined every 2 hours (8 AM to 8 PM) for the presence of a copulatory plug and twice daily (8 AM and 8 PM) for spermatozoa in the vaginal smear. Mated and unmated females were put to death 11 days after copulation or after closure of the vaginal membrane, respectively. Animals without opening of the vaginal membrane were killed on the thirty-sixth day of RU 486 administration.

Hormone assays. The plasma concentrations of progesterone were measured by radioimmunoassay.[7] All samples from each study were measured in one assay; the intraassay coefficient of variation was ≤12%.

Statistical analysis. All results are expressed as mean ± SE. Data were analyzed by means of one-factor analysis of variance. Differences were considered significant at a p level of <0.05.

Results

Study 1: Postcoital administration of RU 486. RU 486, 3 mg/kg/day, completely inhibited nidation (Table I). Only 4 of 10 animals receiving 2 mg/kg/day had implantation sites, a significant reduction ($p = 0.0214$) in the implantation rate (80%) seen in the control group. There were no differences among groups in the number of corpora lutea, suggesting similar ovulatory

Table II. Effects of RU 486 on ovarian activity, mating behavior, and implantation rate

Dose (mg/kg/day)	*No.*	*Cycling animals**	*Cycle length† (days)*	*Progesterone‡ (ng/ml)*	*No. of mated animals*	*Corpora lutea*	*Implantation sites*
0	12	12	15.6 ± 0.3	2.2 ± 0.3	11	42§ (12‖)	32§ (9‖)
1	12	9	16.8 ± 0.5	2.1 ± 0.3	7	28 (8)	7 (2)
2	12	10	15.8 ± 0.8	2.0 ± 0.3	4	22 (9)	0 (0)
3	12	11	13.3 ± 0.4¶	2.7 ± 0.4	5	24 (9)	0 (0)

Treatment was started on day 2 of a nonpregnant cycle and continued until day 11 of the next cycle.

*Number of animals with cyclic vaginal changes. Only these animals were included in the determination of cycle length and progesterone levels.

†Number of days elapsed between two subsequent leukoyctic smears.

‡Mean ± SE on day 8 of the first treatment cycle.

§Total number of corpora lutea or implantation sites.

‖Number of animals with corpora lutea or implantation sites.

¶Significantly different, compared with control group ($p = 0.0002$).

rates in all animals. Progesterone concentrations were similar on postcoital day 7 in all groups but were significantly reduced ($p = 0.004$) on postcoital day 10 in animals receiving RU 486 at a dose of 3 mg/kg/day.

Study 2: Precoital administration of RU 486. Daily administration of RU 486 throughout the estrous cycle prevented cyclic vaginal changes in one to three animals at each dose (Table II). No mating occurred and no corpora lutea were seen in these females.

All other animals exhibited openings of the vaginal membrane associated with typical changes of the vaginal smear. RU 486 at a dose of 1 or 2 mg/kg/day did not affect the length of the cycle, as determined by the number of days elapsed between either two subsequent openings of the vaginal membrane or two subsequent leukocytic smears (Table II). However, the cycle was significantly shortened in animals treated with 3 mg/kg/day as compared with female controls. Progesterone levels on cycle day 8 were similar in all groups (Table II).

One or two animals in each RU 486–treated group had cyclic vaginal opening but lacked corpora lutea (Table II). When present, the number of corpora lutea in each animal decreased significantly with RU 486 administration at a dose of 2 mg/kg/day (2.8 ± 0.2, $p = 0.0031$) and 3 mg/kg/day (2.7 ± 0.3, $p = 0.0031$) compared with vehicle administration (3.5 ± 0.9).

Mating of cycling females was progressively inhibited as the dose of RU 486 was increased, occurring in 92% of the control group, 78% of the animals receiving 1 mg/kg/day, and 40% to 45% of those treated with the two higher doses of RU 486. Copulatory signs were more common in RU 486–treated females with corpora lutea (58%) than in those without corpora lutea (10%).

Nine of 11 mated control animals had implantation sites, as compared with two of seven mated females treated with RU 486, 1 mg/kg/day. RU 486 completely inhibited nidation in the nine mated animals receiving 2 or 3 mg/kg/day.

Comment

Our first study demonstrated that postcoital administration of RU 486, 3 mg/kg/day, completely inhibited implantation in the guinea pig. This antinidatory property of RU 486 probably derives from blockade of progesterone action on the endometrium. In addition, the late luteolytic action of this compound may contribute to the functional deficiency of progesterone induced by RU 486 at the receptor level. Others have shown that progesterone is required for implantation in the guinea pig, because oophorectomy performed within 2 days of mating prevents pregnancy.[4] In addition to its effects on the endometrium and ovary, RU 486 may alter tubal transport and cause the egg to reach the uterine cavity at a time when the endometrium is refractory to implantation. Both accelerated transport of ova as a result of RU 486 treatment[8, 9] and the existence of a short phase of endometrial receptivity to nidation[10] have been reported in the rat. It is also possible that RU 486 prevents implantation in the guinea pig by inhibiting embryonic development. In other rodents, precoital or postcoital administration of RU 486 resulted in the loss of a large number of embryos because of either early expulsion or destruction of eggs. These effects may derive from a direct action of RU 486 on either the reproductive tract of the mother or the cleavage-stage embryo.[8, 11, 12]

In our second study RU 486 administration completely inhibited opening of the vaginal membrane and ovulation in 17% and 28% of the animals, respectively, regardless of the dose used. At doses of 2 or

3 mg/kg/day, the compound also decreased the total number of corpora lutea in each animal. These effects may reflect suppression of gonadotropin secretion, resulting in disrupted folliculogenesis and ovarian steroidogenesis, and may be analogous to the inhibition of follicular development and steroidogenesis seen with follicular phase administration of RU 486 to women.[13, 14] In particular, estrogen plays an important role in the opening of the vaginal membrane[15] and in the initiation of the preovulatory luteinizing hormone surge in the guinea pig.[16]

The length of the estrous cycle decreased when RU 486 was given at a dose of 3 mg/kg/day, an effect similar to the one observed after luteectomy.[17] This may represent an action of RU 486 to reverse the progesterone inhibition of gonadotropin secretion and thus accelerate folliculogenesis and ovulation.

Mating was inhibited in a dose-dependent fashion by preovulatory administration of RU 486. A similar inhibition of sexual behavior was observed with RU 486 administration to oophorectomized, estrogen-primed guinea pigs treated with progesterone.[18] These results, together with the finding that progesterone concentrations rise during estrus, strongly support the concept that estrus is induced by progesterone after adequate estrogen priming.[19-21] This compound also seemed to dissociate estrus from ovulation, because roughly half of the treated animals ovulated but did not mate and a few females mated without ovulating.

Precoital administration of RU 486 prevented pregnancy in 14 of 16 mated guinea pigs. In contrast to the dose-response results seen with postcoital administration of this compound, implantation was completely blocked at a dose of 2 mg/kg/day. This suggests that RU 486 has a cumulative effect when given for a more extended period of time or, alternatively, that it inhibits fertilization. However, the compound does not affect fertilization of the egg in mice.[12]

The antifertility properties of RU 486 observed in the guinea pig may be extrapolated to other species, such as the human, that also require progesterone for the differentiation of the endometrium and implantation.[1] So far, the contraceptive efficacy of RU 486 in women has been assessed by giving the compound at a large single dose in the mid or late luteal phase. Although RU 486 consistently induced menses within 72 hours of its administration in women not at risk for pregnancy,[22, 23] it failed to prevent pregnancy in 18.8% of women having unprotected intercourse.[24] No clinical trials have been conducted to test the ability of RU 486 to interdict implantation when given throughout the cycle.

In summary, our studies show that daily administration of RU 486 can prevent pregnancy in sexually active, ovulatory guinea pigs. These results suggest a novel use for antiprogestin compounds taken as daily contraceptive agents to prevent nidation in women. However, mating behavior and ovarian activity were inhibited in a significant number of RU 486–treated animals. Thus, before a similar contraceptive trial can be conducted in women, the ability of RU 486 to disrupt endometrial maturation without significantly affecting other reproductive function must be evaluated.

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Fetal heart rate response to vibroacoustic stimulation during low and high heart rate variability episodes in late pregnancy

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In 52 women in late pregnancy, the mean durations of transient fetal tachycardia after vibroacoustic stimulation during low fetal heart rate variability (4.8 minutes) and high fetal heart rate variability (6.3 minutes) were similar. The fetal heart rate continued with high variability in all cases, suggesting that the fetus did not return to its prestimulation state after vibroacoustic stimulation during quiescence. In 10 women, the duration of high variability after vibroacoustic stimulation during low fetal heart rate variability was significantly shorter (mean, 22 minutes) than the preceding (mean, 36 minutes) or subsequent (mean, 43 minutes) high-variability components of complete rest activity cycles. In another 10 women, the duration of high variability after vibroacoustic stimulation during high fetal heart rate variability was similar to preceding and subsequent high-variability episodes. In these 20 women, the next complete rest-activity cycle after vibroacoustic stimulation was not different in duration to the complete cycle recorded on the previous day. (AM J OBSTET GYNECOL 1991;165:86-90.)

Key words: Vibroacoustic stimulation, fetal heart rate variability, fetal behavioral cycles

The normal baseline fetal heart rate (FHR) in late pregnancy is characterized by alternating episodes of low and high FHR variability[1, 2] that accompany quiet and active behavioral states in the fetus.[3] Such variability changes have been used to study fetal behavior in late pregnancy,[4, 5] during labor,[6] and in the newborn.[7, 8] In the term fetus, vibroacoustic stimulation during fetal quiescence resulted in a transient increase in baseline FHR (tachycardia) for up to 15 minutes.[9-11] with an increase in the frequency and size of accelerations, associated with an increase in fetal movements, for up to 1 hour.[9, 12, 13] The extent to which this abrupt change in the level of arousal,[14] likened to a startle response,[15] interrupts the natural cycle of fetal behavioral states is unknown. To assess the degree of fetal disturbance after vibroacoustic stimulation we compared the FHR response to stimulation during low FHR variability with the response obtained to stimulation during high FHR variability. We also compared complete cycles of low and high FHR variability episodes

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before vibroacoustic stimulation with the type and duration of poststimulation variability as well as the next complete cycle of low and high FHR variability episodes after vibroacoustic stimulation.

Material and methods

Fifty-two women with singleton pregnancies between 36 and 42 weeks' gestation, all with a cephalic presentation, were studied after antenatal admission to Queen Charlotte's and Chelsea Hospital. The reasons for admission were postmaturity (8), suspected intrauterine growth retardation (6), mild pregnancy-induced hypertension (10), social problems (3), placenta previa (6), planned elective lower segment cesarean section (11), and other reasons (8). Only women who reported normal fetal movements were included in the study. No study participants were taking medication or were smokers. Informed consent was obtained from each woman and the study was approved by the hospital ethics committee.

Studies were performed at the same time each morning to exclude the effects of diurnal rhythms. The FHR was recorded with a pulsed Doppler ultrasonographic transducer and an FM7 fetal monitor (Oxford Sonicaid, Chichester, United Kingdom). The mother was semirecumbent and in a quiet room. After 10 minutes of FHR record a vibroacoustic stimulator (Corometrics) was placed on the maternal abdomen over the fetal vertex and activated for 5 seconds.[9, 12, 13] In 25 cases vibroacoustic stimulation was applied after 10 minutes of low FHR variability and in 27 cases it was applied after 10 minutes of high FHR variability. The FHR record was continued until the poststimulation episode of FHR variability changed. In a subgroup of 20 women a long FHR record was made without vibroacoustic stimulation on the first morning to obtain a complete behavioral cycle, as indicated by consecutive low and high FHR variability episodes. The next morning, at the same time, vibroacoustic stimulation was applied after 10 minutes of low FHR variability in 10 women and after 10 minutes of high FHR variability in the other 10 women. These FHR records were then continued until the next complete cycle of low and high FHR variability episodes was obtained after the poststimulation episode.

Low FHR variability episodes were defined as periods of at least 5 minutes' duration with a stable FHR baseline, decreased baseline (long-term) variability (amplitude less than 10 beats/min) and few or no FHR accelerations (FHR pattern A associated with fetal state 1F, quiescence[3]). High FHR variability episodes were identified when the baseline FHR variability was greater than 10 beats/min amplitude with accelerations (FHR pattern B associated with fetal state 2F, activity[3]). Episodes included in the study were complete in that they were preceded and followed by a change from one level of variability to another. All durations were determined from the FHR trace and measured to the nearest whole minute. An acceleration was defined as an abrupt rise in FHR of more than 15 beats/min, returning toward the baseline again after reaching a peak. Tachycardia was defined as an increase in baseline FHR of more than 15 beats/min for longer than 1 minute.

The amplitude and duration of the initial tachycardia in response to vibroacoustic stimulation and the nature and duration of the variability episode immediately after vibroacoustic stimulation during low FHR variability were compared with the effects of vibroacoustic stimulation during high FHR variability with the unpaired Student t test. In the subgroup of 20 women the mean durations of the prestimulation episodes of low and high FHR variability in a complete cycle were compared with the duration of poststimulation variability after vibroacoustic stimulation and with the episodes in the next complete cycle of FHR variability episodes with the Student paired t test and the Wilcoxon matched-pair, signed-rank test. Significance was accepted when $p < 0.05$.

Results

All fetuses stimulated during low FHR variability responded with a transient baseline tachycardia that continued in high variability after returning to the baseline (Fig. 1). Those stimulated during high FHR variability also showed a similar transient tachycardia and then continued in high variability. The amplitude of the tachycardia rarely exceeded the size of the largest acceleration noted during preceding or subsequent high FHR variability and was similar irrespective of the variability at the time of stimulation (Table I). The mean duration of the tachycardia and the mean duration of high variability heart rate immediately after the tachycardia were similar whether vibroacoustic stimulation was applied during low or high FHR variability (Table I). The baseline FHR after the tachycardia was not significantly different from the baseline rate before stimulation, despite the increased variability and number of accelerations (Fig. 2).

Of the 20 women with long FHR records before vibroacoustic stimulation, the mean durations of high and low FHR variability episodes were not significantly different between the 10 stimulated in low FHR variability and the 10 stimulated in high FHR variability (Table II). The mean duration of high variability (22.8; SD, 11.6; range, 9 to 46 minutes) that followed vibroacoustic stimulation during low FHR variability ($n = 10$) was significantly shorter than the high variability episodes that contributed to the complete cycles in the same women before (36.4; SD, 14.9 minutes; $p < 0.01$) and after (42.8; SD, 20.1 minutes; $p < 0.05$) stimulation. However, the mean duration of high vari-

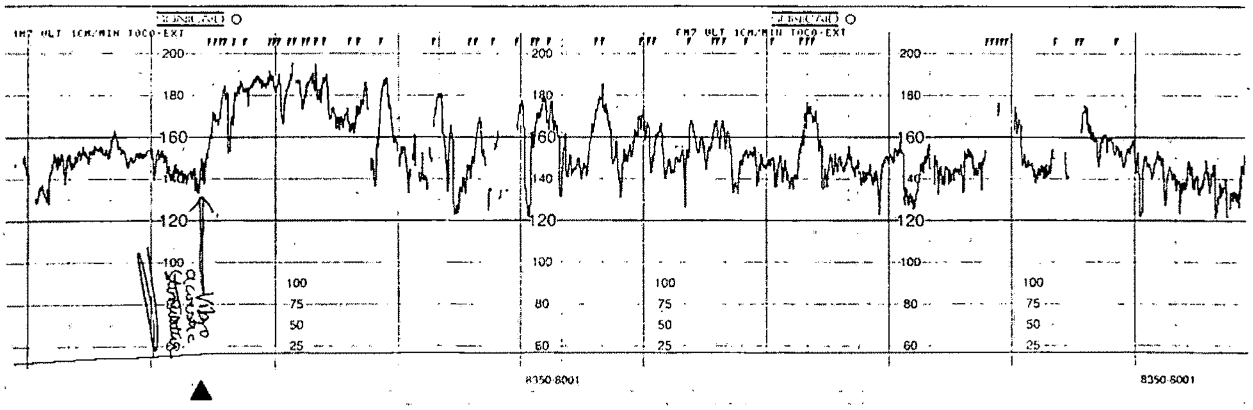

Fig. 1. Tachycardia of 5 minutes' duration associated with multiple fetal movements (state 4F, awake) followed by continuation of high variability and movements (state 2F, active) in response to vibroacoustic stimulation (*large triangle* below the record) during low FHR variability (state 1F, quiet). Maternally perceived fetal movements are indicated by *small triangles* along the top of the record. *Vertical bars*, 3 cms.

Table I. FHR response to vibroacoustic stimulation

	No.	*Mean*	*SD*	*Minimum*	*Maximum*
Amplitude of tachycardia (beats/min)					
VAS in low FHRV	25	33.4	7.87	15	50
VAS in high FHRV	27	37.4	9.24	20	60
Duration of tachycardia (min)					
VAS in low FHRV	25	4.8	3.02	1	14
VAS in high FHRV	27	6.3	3.24	2	15
Subsequent high FHRV (min)					
VAS in low FHRV	25	37.6	25.7	9	120
VAS in high FHRV	27	51.6	30.9	10	109

VAS, Vibroacoustic stimulation; *FHRV,* fetal heart rate variability.

ability (42.4; SD, 28.2; range, 16 to 109 minutes) after vibroacoustic stimulation during high FHR variability ($n = 10$) did not differ from the prestimulation (50.5; SD, 27.3 minutes) and poststimulation (40.4; SD, 18.2 minutes) high variability episodes in these women. In all 20 cases, the mean durations of low and high FHR variability episodes before stimulation were not significantly different from the mean durations of those in the next complete FHR variability cycle that occurred subsequent to the response episode (Table II). The results of analyses with the Student t test and the Wilcoxon matched-pair test were similar. There was no significant difference in the amplitude of accelerations during the spontaneous high FHR variability episodes before and after stimulation.

Comment

The immediate response to vibroacoustic stimulation by all cases in this study was an abrupt rise in baseline FHR. We consider this a transient tachycardia because it lasted longer than the duration of an acceleration although it always returned to the preceding baseline level within 15 minutes. Previous reports have described this initial response as a significant rise in baseline FHR,[9, 10, 13] a significant increase in mean duration of accelerations,[16] and a tachycardia.[11] We found that the mean amplitude of this response often took the rate above 150 beats/min, the recently proposed upper limit of normal for the FHR,[17] for between 1 and 15 minutes and so we believe that the description of tachycardia is more appropriate. However, contrary to one previous report[16] we found that the mean amplitude of this tachycardia was not greater than the largest of spontaneous accelerations that occurred either before after stimulation. FHR accelerations are known to be associated with spontaneous fetal movements[18] and this supports the idea that the transient tachycardia after vibroacoustic stimulation is associated with an increase in fetal movements, consistent with the awake fetal state.[3] However, because this may be brief (Fig. 2), some methods of analysis have failed to show an increase in fetal movements.[10, 12] One study with ultrasonography found a fetal startle response each time after vibroacoustic stimulation.[15] All fetuses in our study responded to vibroacoustic stimulation.

We found the duration of transient tachycardia after vibroacoustic stimulation during low FHR variability to be similar to that reported previously.[9, 10] It is of interest that both amplitude and duration of the tachycardia after stimulation during high FHR variability were sim-

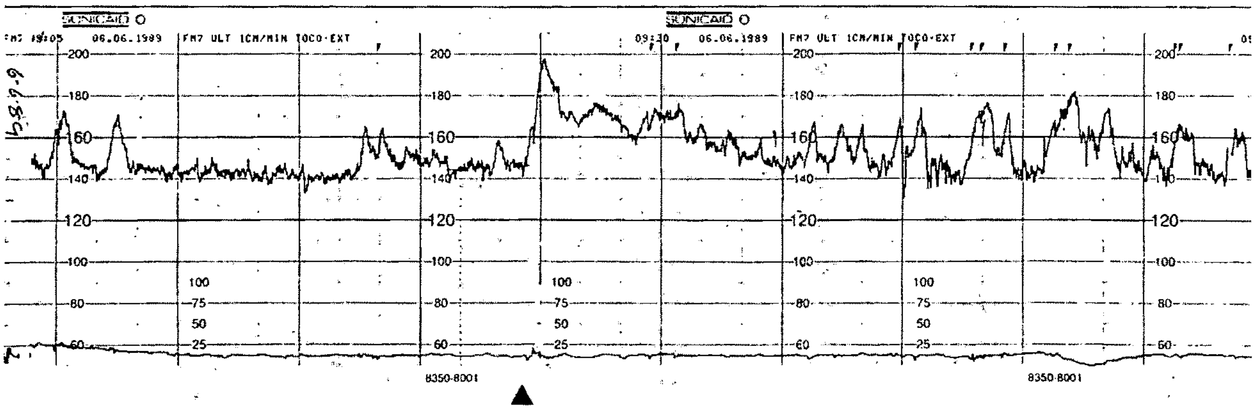

Fig. 2. Contrast between FHR variability, number and size of accelerations, and number of fetal movements during fetal quiet state before vibroacoustic stimulation (*large triangle* below the record) with the poststimulation appearance. Maternally perceived fetal movements are indicated by *small triangles* along the top of the record. *Vertical bars,* 3 cms.

Table II. Cycles of FHR variability episodes before and after vibroacoustic stimulation

	Before VAS				*After VAS*			
	Mean	*SD*	*Minimum*	*Maximum*	*Mean*	*SD*	*Minimum*	*Maximum*
VAS in low variability								
Low FHRV episodes	11.6	6.5	5	25	10.7	8.8	5	28
High FHRV episodes	36.4	14.9	16	54	42.8	20.1	14	90
Complete cycle	48.0	16.1	21	72	53.5	18.9	30	96
VAS in high variability								
Low FHRV episodes	12.8	5.1	8	24	10.7	4.0	5	18
High FHRV episodes	50.5	27.3	22	120	40.0	18.2	15	70
Complete cycle	63.3	25.6	36	128	50.7	17.9	23	80

All durations in minutes.
VAS, Vibroacoustic stimulation; *FHRV,* fetal heart rate variability.

ilar to that after vibroacoustic stimulation during low FHR variability, indicating that this aspect of the fetal response to vibroacoustic stimulation did not depend on the preexisting FHR variability. Devoe et al.[19] also reported that all fetuses responded to vibroacoustic stimulation with a brief tachycardia irrespective of the preexisting state. The sudden and abrupt change in FHR has led to the suggestion that the fetus actually wakes up (state 4F[3])[19] and this is supported by the fact that the mother feels vigorous fetal movements[11, 12, 15] associated with the sustained but transient tachycardia (FHR pattern D according to Nijhuis et al.[3]). The initial FHR response to vibroacoustic stimulation can therefore be explained by the abrupt transition into a transient period of wakefulness; our findings confirm that this response also follows vibroacoustic stimulation when applied during high FHR variability.

It is generally accepted that episodes of high FHR variability represent the active sleep state in the fetus (state 2F) and these alternate with episodes of low FHR variability, representing periods of quiet sleep (state 1F) to characterize most of the continuous FHR record after 36 weeks' gestation.[1, 4, 20] The duration of the second component of the fetal response to vibroacoustic stimulation applied in low FHR variability, namely the continuation in high variability, has not been previously reported. Persistance of body movements and a raised computer-derived baseline FHR for up to 1 hour have been described.[12, 13] We found that all fetuses, whether stimulated during low or high variability, continued in high variability after vibroacoustic stimulation. Thus after the initial response to stimulation, all fetuses remained in state 2F and the duration was similar irrespective of the variability at the time of vibroacoustic stimulation. Therefore it would seem that when stimulated into wakefulness from active sleep the fetus returned to this state but when stimulated into wakefulness from quiet sleep the fetus did not return to the quiet sleep state but remained in active sleep. Thus alteration of fetal behavioral states is greater after vibroacoustic stimulation during low FHR variability.

The 20 women in the subgroup studied on 2 consecutive days were selected because they had a complete cycle of FHR episodes within the 120-minute record made on the first occasion. Therefore the mean durations of consecutive low and high FHR variability episodes shown in Fig. 2 are shorter than some previous reports[1, 6] but are similar to others derived from shorter records.[18] However, each of these 20 women acted as her own control for the comparison between complete cycles before and after vibroacoustic stimulation and for the comparison between high variability episodes before and after with the duration of high variability that followed the transient tachycardia. Our results show that vibroacoustic stimulation during high variability resulted in a duration of poststimulation high variability similar to the durations of high variability episodes during behavioral cycles before and after stimulation. However, the duration of high variability after vibroacoustic stimulation during low variability was significantly shorter than the prestimulation and poststimulation high variability components of complete rest-activity cycles, suggesting that the active fetal state after the abrupt transition from quiescence to wakefulness may not be the same as the spontaneous episodes of fetal activity that contribute to rest-activity cycles. The next complete spontaneous behavioral cycle was of similar duration to the cycle recorded on the previous day, indicating that the disruption of fetal behavior did not extend beyond the stimulated cycle.

In conclusion, our findings agree with previous studies that indicate that vibroacoustic stimulation during low FHR variability produces an abrupt change from quiet to active fetal state through a transient period of wakefulness. In addition, we have shown that the fetal response to vibroacoustic stimulation during high FHR variability is a similar transient period of wakefulness before resumption of the preexisting active state. Although we have shown that disruption of fetal rest-activity cycles does not extend beyond the stimulated cycle, we do not believe that this implies safety. The value of vibroacoustic stimulation as a means of antenatal fetal investigation needs to be investigated in the form of a randomized trial relating to neonatal outcome and not solely to the production of a reactive FHR record, which it does quite effectively.[21]

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Elective versus emergency cesarean hysterectomy cases in a residency program setting: A review of 129 cases from 1984 to 1988

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A retrospective study was performed to compare elective versus emergency cesarean hysterectomy in a residency program. We noted the following statistically significant differences between emergency and elective cases: (1) Estimated blood loss (mean ± SD) for emergency and elective cases was significantly different at 1495 ± 772 and 875 ± 396 ml ($p = 0.001$). (2) Operative time in emergency cases was significantly longer than in elective cases (105 ± 38 vs 83 ± 27 minutes, $p = 0.003$). (3) The incidence of transfusion was 68% in our emergency cases and 14.6% in the elective series ($p < 0.05$). (4) Febrile morbidity in our series was 40% in emergency cases and 18.3% in elective cases ($p < 0.05$). Our series was comparable to previous series reported. Our experience suggests that cesarean hysterectomy can be performed safely in a residency training program when done as an elective procedure in the well-prepared patient. (AM J OBSTET GYNECOL 1991;165:91-4.)

Key words: Cesarean hysterectomy, operative morbidity

Emergency hysterectomy has been accepted as a lifesaving procedure for patients with bleeding at the time of cesarean section. Elective cesarean hysterectomy is considered controversial by many because of concern about increased bleeding, transfusion, and urologic complications. Cesarean hysterectomy may be an alternative to cesarean section and tubal ligation, particularly for those patients with a history of menorrhagia, dysmenorrhea, leiomyomas, or other problems occurring before pregnancy that predict further gynecologic surgery. Cesarean hysterectomy is not offered to patients who are not undergoing a cesarean section for indicated reasons.

This paper reports on emergency versus elective cesarean hysterectomy in a residency program setting. Our outcomes were compared with those of previous studies reported in the literature.

Material and methods

A retrospective chart analysis was undertaken on all cases of cesarean hysterectomy performed between 1984 and 1988. Operations included for review were those performed after 28 weeks of gestation and after abdominal delivery. Hysterectomies performed after vaginal delivery were excluded.

The operations were classified as either *elective indicated* or *emergency*; the latter were performed as a lifesaving procedure to prevent exsanguination in cases of profuse bleeding. Elective cases are considered to be those in which the procedure was planned and performed on patients in whom hysterectomy and cesarean delivery were indicated. Patients in whom the procedure was used to treat severe cervical dysplasia were evaluated by colposcopy during the pregnancy. Patients with leiomyomas that were present and symptomatic during the nonpregnant period were considered elective indicated cesarean hysterectomy.

Our policy was to offer this procedure in a clinic setting, before the stress of labor occurred and where an informed consent could be obtained after the patient had discussed the decision with her partner. These patients were given full explanations of the risks and benefits of this operation as opposed to cesarean section and tubal ligation. A staff physician supervised the cases, but the operations were performed by third- and fourth-year residents. The procedure used was the clamp-cut-and-tie-later technique as advocated by Plauché.[1] Prophylactic antibiotics were used in 82% of cases. Febrile morbidity was defined as two temperatures >38° C that occurred at least 6 hours apart (excluding the first 24-hour postoperative period). Change in hematocrit was calculated as preoperative hematocrit minus the last hematocrit before discharge in patients who did not need transfusion. Blood loss estimations were made by the anesthesia personnel in each case. These procedures were performed at the University Medical Center and at Earl K. Long Hospital, both of

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Table I. Comparison of demographic parameters between elective and emergency cesarean hysterectomy

Parameter	*Emergency (n = 22)*	*Elective (n = 107)*	*p Value*
Age (yr)	28.1 ± 5.8	31.0 ± 5.6	0.029
Gravidity	4.0 ± 1.8	4.2 ± 2.2	0.761
Parity	2.4 ± 1.5	3.1 ± 2.2	0.167
Gestational age (wk)	33.5 ± 5.0	38.4 ± 1.9	0.001
Previous cesarean section	1.0 ± 1.1	1.1 ± 1.0	0.763
Apgar score			
1 min	5.5 ± 3.2	8.3 ± 1.2	0.001
5 min	6.6 ± 3.2	9.0 ± 0.4	0.001

Table II. Indications for emergency cesarean section hysterectomy

Indication	*No.*	*Percentage of total*
Hemorrhage	8	36
Uterine rupture*	6	27
Uterine incision dehiscence†	3	14
Placenta previa	2	9
Placenta accreta	2	9
Abruptio placentae	1	5

*At 25, 29, 31, 32, 33, 39 weeks' gestation.
†At 39 and 40 weeks' gestation.

which are affiliated hospitals of Louisiana State University Medical School in New Orleans.

Data are reported as mean ± SD. Groups were compared by unpaired Student *t* tests, with $p < 0.05$ considered significant.

Results

During the 5-year study period there were 33,963 deliveries, 4891 of which were cesarean sections; this resulted in a cesarean section rate of 14.2%. A total of 129 cesarean hysterectomies were performed during the study period; there were 107 elective cases and 22 emergency cases.

Gestational age at delivery was significantly higher for emergency cases than for elective cases (33.5 ± 5.0 and 38.4 ± 1.9 weeks, respectively, $p = 0.001$). Apgar scores at 1 minute of life were 5.5 ± 3.2 and 8.3 ± 1.2 for emergency and elective cases, respectively ($p = 0.001$). Apgar scores at 5 minutes were 6.6 ± 3.2 for emergency cases and 9.0 ± 0.4 for elective cases ($p = 0.001$). Demographic data are summarized in Table I.

Indications for emergency cases are summarized in Table II. Hemorrhage, uterine rupture, and uterine incision dehiscence were the most common indications for emergency cesarean hysterectomy. The majority of cases of uterine rupture, which were associated with fetal distress, occurred before term, whereas three cases of uterine dehiscence (uterine scar separation found incidentally at the time of repeat cesarean section) occurred at term in this series. In all cases the surgeons were able to remove the cervix at the time of hysterectomy.

Table III. Indications for elective indicated cesarean section hysterectomy

Indication	*No.*	*Percentage of total*
Undesired fertility and history of dysmenorrhea and menorrhagia	50	45
Leiomyomas	17	16
Severe cervical dysplasia	15	14
Undesired fertility	11	10
Failed bilateral tubal ligation	7	6
Chronic pelvic pain	4	4
Placenta previa	3	3
Symptomatic descensus	2	2

Table IV. Operative parameters: elective versus emergency cesarean hysterectomy

Parameter	*Emergency (n = 22)*	*Elective (n = 107)*	*p Value*
Operating time (min)	105 ± 38	83 ± 27	0.003
Estimated blood loss (ml)	1495 ± 772	875 ± 396	0.001
Hospital stay (days)	5.5 ± 2.3	4.2 ± 1.8	0.004

The most common indication for elective cesarean hysterectomy was previous cesarean delivery with undesired future fertility, as well as a history of dysmenorrhea and menorrhagia. Other indications included symptomatic leiomyomas, cervical dysplasia, and previous failed tubal ligation. The percentages of each indication for elective cesarean section are given in Table III.

Operative time in emergency cases was significantly longer than in elective cases (105 ± 38 vs 83 ± 27 minutes, $p = 0.003$). Estimated blood loss for emergency and elective cases was 1495 ± 772 and 875 ± 396 ml, respectively ($p = 0.001$). Three elective and four emergency cases required preoperative transfusion. Mean change in hematocrit in those elective cases that did not require transfusion was −3.7% ± 3.6%. Both units of transfusion and length of hospital stay were greater in the emergency cases. All operative parameters are displayed in Table IV.

No maternal deaths occurred in patients undergoing cesarean hysterectomy. Complications at the time of cesarean hysterectomy are summarized in Table V. Infectious morbidity included vaginal cuff cellulitis, pneumonia, and urinary tract infection. Reexploration after cesarean hysterectomy occurred in one emergency case as a result of postoperative bleeding at the vaginal cuff area, which was controlled with suture li-

Table V. Complications of cesarean hysterectomy

Complication	Emergency ($n = 22$) No.	Emergency ($n = 22$) %	Elective ($n = 109$) No.	Elective ($n = 109$) %
Febrile morbidity	4	18	12	11
Vaginal cuff cellulitis	5	23	8	7
Pneumonia	0	0	1	1
Urinary tract infection	1	4	2	2
Postoperative ileus	1	4	1	1
Broad ligament hematoma	0	0	1	1
Vaginal cuff bleeding	0	0	2	2
Wound infection	0	0	2	2
Reexploration	1	4	0	0
Cystotomy	0	0	1	1
Transfusion	15	68	16	15

Table VI. Procedures performed at the time of cesarean hysterectomy

Procedure	Emergency ($n = 22$)	Elective ($n = 107$)
Unilateral salpingectomy and oophorectomy	1	22
Bilateral salpingectomy and oophorectomy	5	7
Appendectomy	3	14
Ventral hernial repair	0	1
Cystotomy repair	0	1

Table VII. Comparison with recent studies for elective cesarean hysterectomy

Factor	This study	Barclay et al.[2]	Plauché et al.[3]	Plauché et al.[1]
Estimated blood loss (ml)	875	—‡	981	—‡
Transfusion (%)	14.7	19.9	50	11.8
Time of operation (min)	83	86	126	65
Febrile morbidity (%)	18.3	31	26.6	30.5

*Series at Louisiana State University.

†Private patients.

‡Statistics not available.

gation. Other complications are also listed and compared in Table V.

There were several patients who underwent other procedures at the time of cesarean hysterectomy. Unilateral or bilateral salpingectomy and oophorectomy were the most common procedures. Unilateral procedures were performed in cases of bleeding ($n = 12$) or in the presence of adnexal pathologic conditions ($n = 4$). The indication for bilateral salpingectomy and oophorectomy was advanced patient age, with a mean age of 41 ± 1.5 years. Appendectomies were performed when a pathologic condition was present and was confirmed microscopically. Procedures performed at the time of cesarean hysterectomy are shown in Table VI.

Comment

Few obstetricians trained after 1980 have experience in performing cesarean hysterectomy. When the need arises for emergency hysterectomy at the time of cesarean section, physicians are faced with a difficult procedure and an unstable patient.

Before the 1940s the major indications for emergency cesarean hysterectomy were hemorrhage and uterine pathologic conditions such as a ruptured uterus. During the 1960s the indications for cesarean hysterectomy were expanded to include elective sterilization. Indications for cesarean hysterectomy in our study were not different from those reported in recent series.[1-3] Pregnancy after tubal ligation at cesarean section has been reported to occur more frequently than when tubal ligation is done as an interval procedure. The failure rate ranges from 0% for the Uchida[4] and Irving[5] procedures to 1.75% for the Pomeroy procedure.[6,7] These failure rates are related to procedure type and technique.

Table VII compares our series to those of Plauché et al.[1-3] and Barclay et al.[2] The mean operating time of elective procedures in our series was 83 ± 27 minutes. Barclay et al.[2] reported a mean operative time in cesarean section and tubal ligation as 51.1 ± 14.5 minutes. In the cesarean hysterectomy series of Plauché et al.,[1] in which the same surgeons did all procedures, the mean operative time was 65 minutes. We feel that the operative time is not a limiting factor when the surgeons are experienced in the procedure.

The mean blood loss in our series was 875 ± 396 ml. For those who did not need transfusion, the change from preoperative to discharge hematocrit was only −3.7% ± 3.6%. We do not feel that excessive blood loss is a contraindication to cesarean hysterectomy performed by surgeons trained in the procedure.

The incidence of transfusion in our elective series was 14.6% as compared with the elective series of Barclay et al.[2] and Plauché et al.,[1] which were 19.9% and 11.8%, respectively. The lower transfusion rate in the series of Plauché et al. might have been related to the experience level of the surgeons or to the higher presurgery hematocrits of the private patients. In our series the mean preoperative hematocrit of those requiring transfusion was 32.6% ± 5.8%, as compared with 35.9% ± 3.0% in patients who did not need transfusion ($p = 0.001$). This lower starting hematocrit may explain the greater risk of transfusion in this group. The incidence of transfusion in cesarean section is reported as 1% to 6%[8]. The risk of illness from transfusion in elective procedures could be reduced by allowing autologous donations. Recently, we have begun to allow autologous donation in our department for patients anticipating elective cesarean hysterectomy. We recommend that those patients who anticipate cesarean hysterectomy donate 2 units of blood before delivery.

The hospital stay for elective cesarean hysterectomy in this series was 4.2 ± 1.8 days, which is comparable to the hospital stay for cesarean section and tubal ligation.[9] In an indicated hysterectomy, we save the patient the risk of undergoing a second procedure and anesthesia. In the series of Bukovsky et al.,[9] in which hysterectomy was used for sterilization, only one patient needed further gynecologic surgery, as opposed to 49 additional procedures required by 86 matched patients who underwent cesarean section and tubal ligation. Ten of these 86 patients later underwent a hysterectomy. Cesarean hysterectomy may help avoid further surgery in those cases in which hysterectomy is indicated at the time of cesarean section.

Febrile morbidity in our series was 18.3% in elective cases. The series of Barclay et al.[2] had a febrile morbidity of 31%, but this was before the use of prophylactic antibiotics. The private and residency series of Plauché et al.[1, 3] reported incidences of 26.6% and 30.5%, respectively, for febrile morbidity. The incidence of febrile morbidity in cesarean section alone has been reported to range from a low of 5% to a high of approximately 85%, with a mean of 30% to 40%.[10] Cesarean hysterectomy in our series had a lower febrile morbidity rate than did cesarean section alone.[10] This may be because cesarean hysterectomy removes the site of endomyometrial infection.

Urologic injuries have been reported as a complication in other series of elective cesarean hysterectomy. The incidence of urologic injuries after cesarean hysterectomy ranges between 0.3% and 0.9%.[11] Plauché et al.[1] reported two cases of cystotomy and another unrecognized bladder injury with subsequent fistula development in 108 cases of cesarean hysterectomy. Haynes and Martin[12] reported 11 cystotomies and one fistula in 149 cesarean hysterectomies. In our series there was only one incidental cystotomy in a patient with two previous cesarean sections. This injury was repaired without complication. The lower incidence of bladder injury in our series may be related to our adherence to cautious bladder reflection at the time of cesarean section. Our practice was to test the bladder with sterile solution in any cases of suspected bladder injury.

The performance of other procedures at the time of cesarean hysterectomy is determined by pathologic conditions noted at the time of surgery and by the desires of the patient. Oophorectomy may be indicated in those with adhesive disease of the adenexa or as a result of the patient's age. Oophoropexy can be useful in preventing dyspareunia.

Our series suggests that cesarean hysterectomy can be a safe procedure when performed electively in the well-prepared patient. Although the incidence of transfusion is higher than that of cesarean section alone, the benefits to the patient who has an indication for hysterectomy may outweigh the risks of undergoing repeated surgery and anesthesia at a later date. As the rate of cesarean section increases, we must be aware of the options available to women who are undergoing a repeat cesarean section. Surgeons with the required skill can offer a cesarean hysterectomy at the time of repeat cesarean section to those patients with an indication for hysterectomy. Performing elective cesarean hysterectomy prepares the obstetrician for the more difficult procedure of emergency cesarean hysterectomy.

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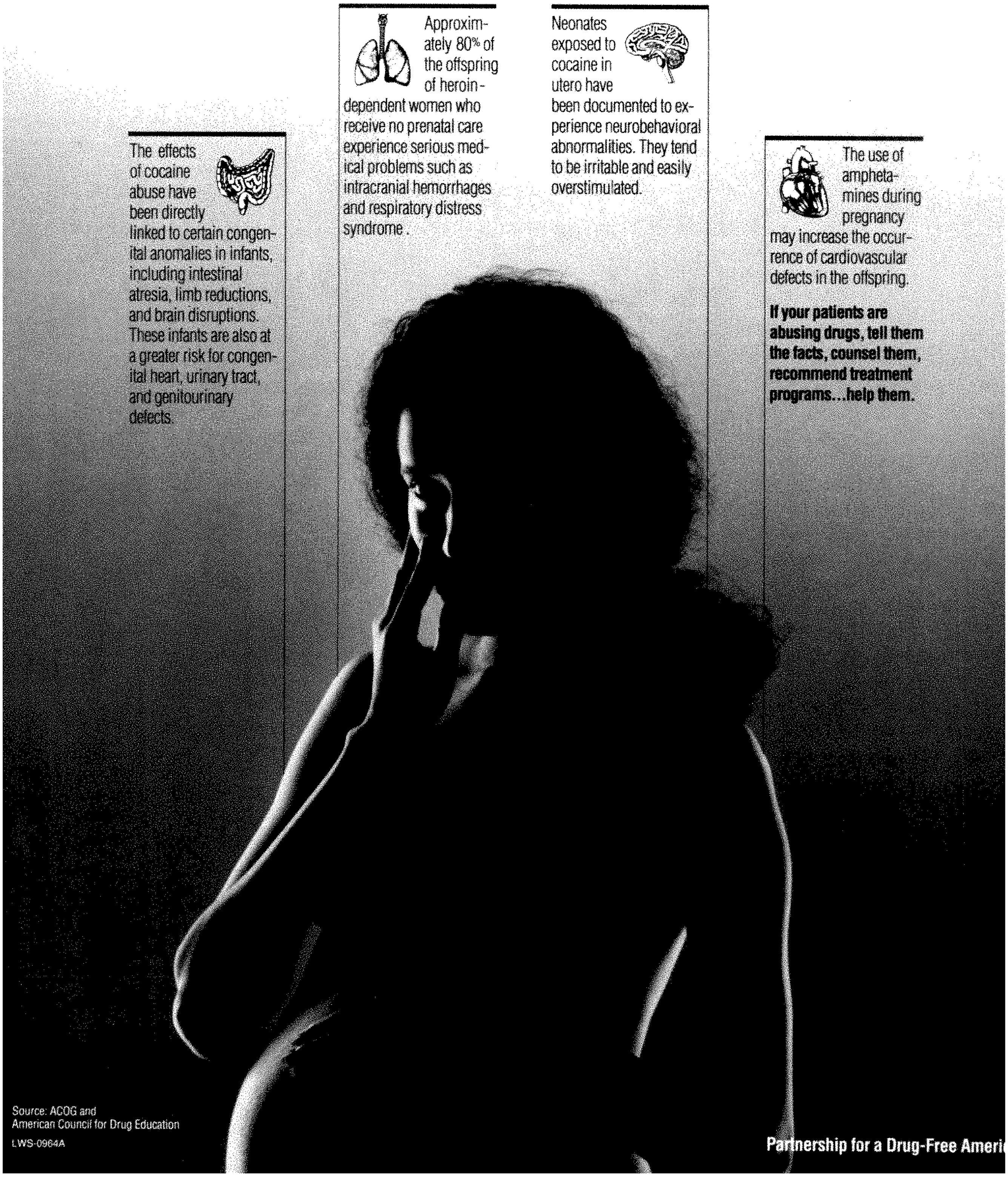

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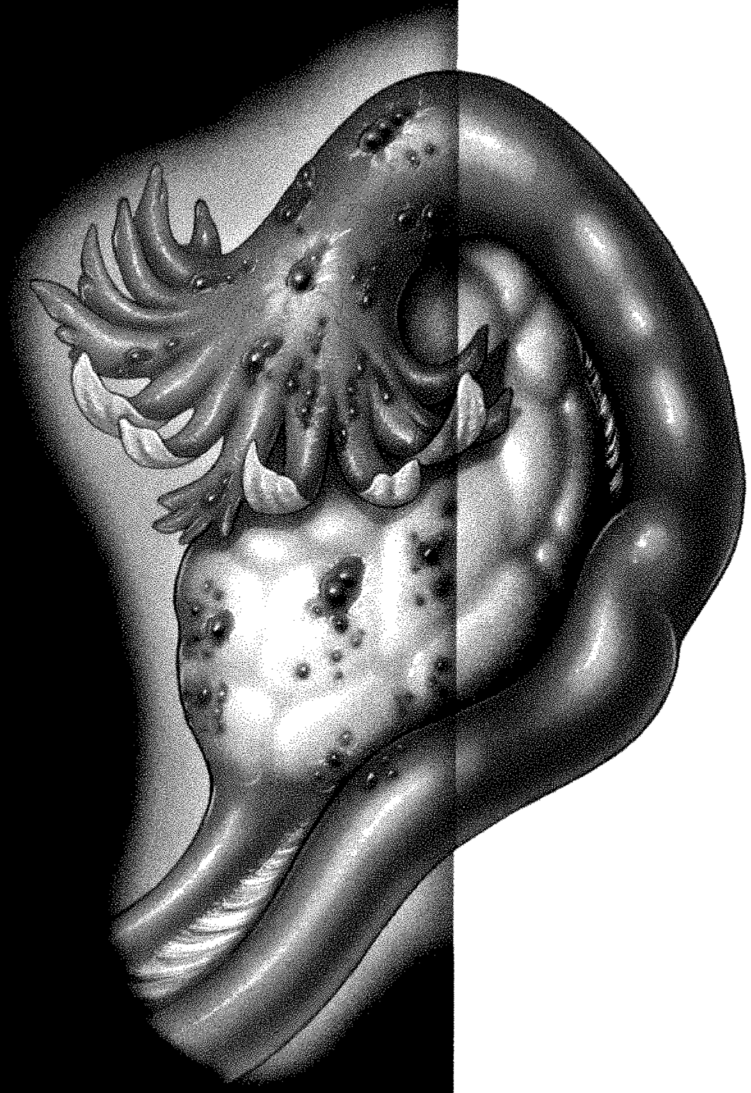

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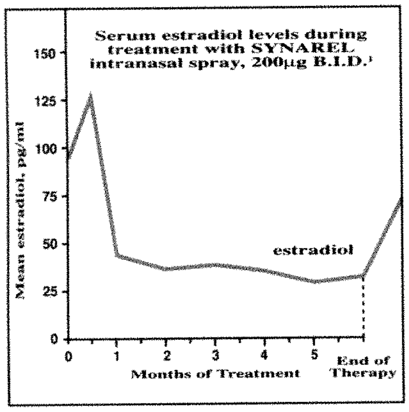

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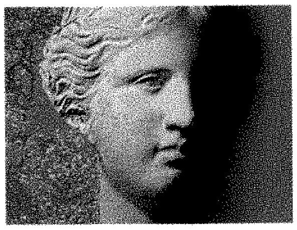

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CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.
WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.
When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.
PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.
Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.
Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.
Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.
Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.
Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.
Pediatric Use: Safety and effectiveness in children have not been established.
ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.
Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.
Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.
Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.
Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (± SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.
Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.
OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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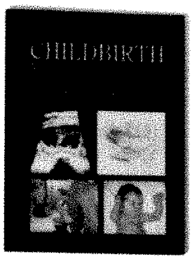

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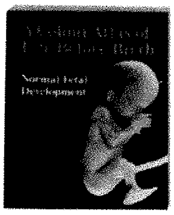

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Transcervical endoscopic verification of congenital anomalies in the second trimester of pregnancy

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Transcervical endoscopy was used to confirm prenatally diagnosed anomalies before elective termination of pregnancy. A complete anatomic survey was accomplished in 10 (70%) of 14 cases studied. Information was added to the ultrasonographic diagnosis in two cases, which changed the diagnosis in one fetus. (Am J Obstet Gynecol 1991;165:95-7.)

Key words: Prenatal diagnosis, endoscopy, genetic counseling

The widespread use of diagnostic ultrasonography in the prenatal diagnosis of structural anomalies allows the option of pregnancy interruption. It is imperative to confirm these suspicions and to evaluate the fetus for subtle defects so that accurate patient counseling can be offered regarding future pregnancies. Whereas a discrete isolated structural defect probably would not be missed, the addition of multiple defects might suggest a genetic syndrome with a higher recurrence rate.

Dilatation and evacuation and prostaglandin induction are commonly used for termination in the second trimester of pregnancy and often render the specimen unsatisfactory for pathologic examination.

In this study we evaluated the utility of transcervical endoscopy in the second trimester to visualize the fetus before the termination of pregnancy in an attempt to confirm prenatally suspected congenital anomalies.

Methods and material

Fourteen patients underwent endoscopic procedures before termination of pregnancy at either The University of Florida Health Science Center at Jacksonville or Yale–New Haven Hospital. Indications for termination included suspicion of a congenital anomaly on ultrasonography (nine patients), aneuploidy detected by amniocentesis (two patients), and three elective terminations. Gestational ages ranged from 14 to 19 menstrual weeks.

Informed consent was obtained under a protocol by the Human Investigation Committee. The endoscopic technique used was similar to that previously reported.[1] Rigid endoscopes (Karl Storz, Culver City, Calif.) 30 cm in length with diameters ranging from 2 to 3.5 mm

Table I. Prenatal ultrasonographic diagnosis and endoscopic correlations

Patient No.	*Ultrasonographic diagnosis*	*Gestational age (wk)*	*Additional findings endoscopically*	*Endoscopic visualization*
1	Cystic hygroma	16	None	Limited—bloody field
2	Cystic hygroma	14	None	Complete
3	Cystic hygroma, cleft lip	19	Cleft palate, pterygium, micropenis	Incomplete
4	Anencephaly	15	None	Complete
5	Anencephaly	17	None	Complete
6	Anencephaly	19	None	Complete
7	Encephalocele	19	None	Complete
8	Encephalocele	18	Cleft palate	Complete
9	Uhl's anomaly	14	None	Complete
10	Aneuploidy	14	None	Complete
11	Aneuploidy	18	None	Incomplete
12	Elective termination	14	None	Complete
13	Elective termination	16	None	Incomplete
14	Elective termination	18	None	Complete

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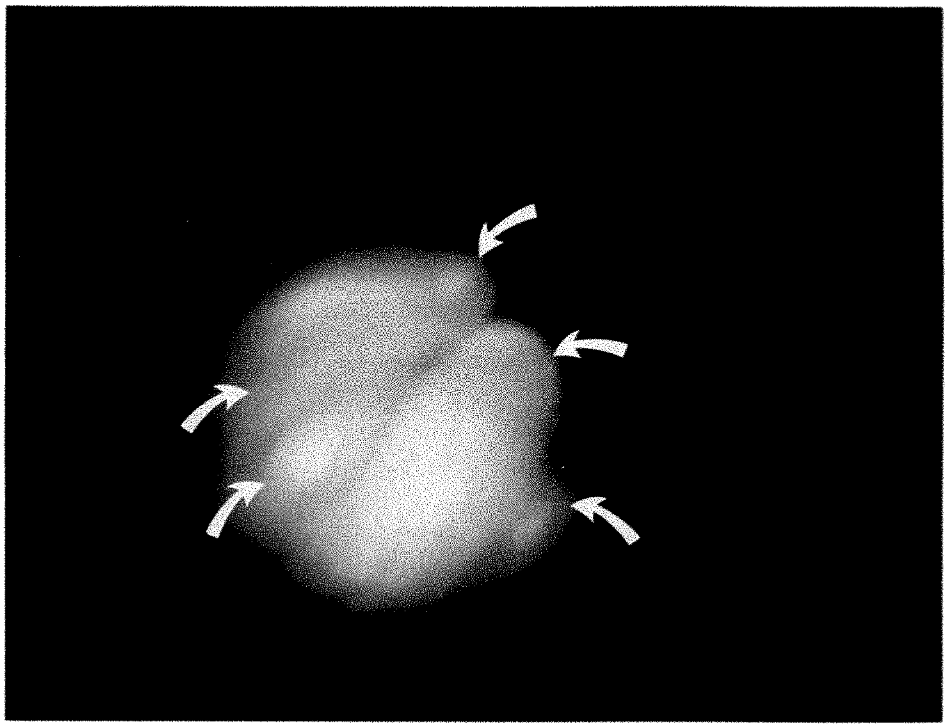

Fig. 1. Endoscopic picture of clinodactyly of hand in fetus at 19 weeks' gestation with multiple pterygium and cystic hygroma. *Arrows* indicate fingers.

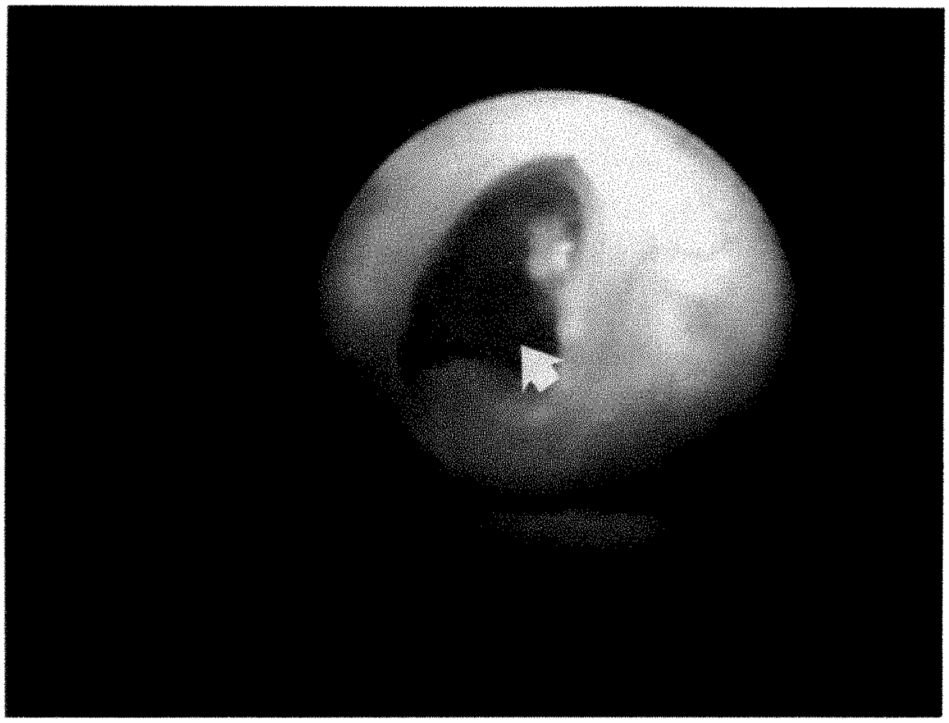

Fig. 2. Endoscopic picture of fetus at 18 weeks' gestation with an encephalocele and cleft palate *(arrow)*. Defect was viewed through endoscope, which was inserted into oral cavity and occupies most of picture.

were introduced through the cervix into the uterine cavity under ultrasonographic guidance. An attempt was made to rupture the opaque chorion while leaving the amnion intact.

Procedures were performed by one of the two operators (M.T.C. or J.W.), and they were viewed via a mini video camera and recorded for subsequent review.

Anesthesia was not induced in the cases in which prostaglandin was used. In cases in which dilatation and evacuation were performed, general anesthesia was often used for the termination. While it is not necessary to dilate the cervix for the endoscopic procedure, laminaria were often placed for the abortion.

A complete anatomic survey of the fetus was attempted; this included visualization of the dorsal and ventral walls, extremities, genitalia, and head and neck, including the oral pharynx. A procedure was considered complete if all but the oral pharynx could be visualized and incomplete if any of the previously mentioned sites were not visualized.

Results

Table I lists the indications for termination of pregnancy, anomalies suspected by ultrasonography, and the results of the endoscopic procedure.

No maternal morbidity or discomfort was reported. Five patients had pregnancy termination by prostaglandin induction and nine by dilatation and evacuation.

Rupture of membranes occurred in all but three cases. While it was our intent to avoid this, it did not detract from the ability to view the fetus in all but one procedure. There was one case in which a partial placenta previa was traversed, which led to a bloody field of view and limited visualization.

The anatomic survey was thought to be complete in 10 cases, helpful but incomplete in three, and inadequate in one. The mouth and oral pharynx could be seen in only three cases. Reasons for the incomplete or difficult examination included breech presentation, rapid leak of amniotic fluid, and a bloody field. The anomalies suspected by ultrasonography were verified in all cases.

In two cases additional information was found at the time of endoscopy; in one of these, the endoscopic findings changed the final diagnosis. One fetus with cystic hygroma had multiple pterygium (Fig. 1), micropenis, and a cleft palate. A cleft palate (Fig. 2) was identified in a fetus with prenatal diagnosis of an encephalocele made by inserting the endoscope into the oral cavity of the fetus.

Comment

Prenatal diagnosis of congenital anomalies is possible in the first[2] and early second[3] trimesters. As ultrasonographic equipment and expertise become readily available, more fetuses will be scanned, and more defects will be detected.

While the prenatal identification of the anomalies is important, accurate genetic consultation with regard to future pregnancies is contingent on complete and verified prenatal data. Although endoscopic visualization does not allow insight into the internal anatomy of the fetus, it does permit an extensive review of the external anatomy. This information was imperative in counseling the third patient in Table I, who was faced with a potential autosomal recessive inheritance in a fetus with a lethal form of multiple pterigium[4] instead of the low recurrence rate for a fetus with isolated cystic hygroma.

Potential difficulties with the procedure include the inability to obtain a complete anatomic survey, rupture of the membranes, and bleeding. While rupture of the membranes occurred often, fluid leakage was often slow and did not detract from the examination. In one case a placenta previa was traversed and bled, which led to poor visualization. Placenta previa might limit the utility of this procedure. We feel that the quality of the examination can be optimized and that risks, such as uterine perforation, can be minimized by ultrasonographic guidance and operator experience.

Transcervical endoscopy differs from transcervical embryoscopy[1] in two ways. The larger size of the fetus in advanced gestation does not allow it to be seen in toto; instead it must be viewed in small segments as it is during fetoscopy. Also, at 13 weeks' gestation the amnion and chorion have often fused, so that it is difficult, if not impossible, to prevent rupture of the membranes. This can be minimized by performing transcervical endoscopy at the earliest possible gestational age and by introducing the scope at an angle more acute than perpendicular to the membranes. The high incidence of ruptured membranes currently precludes use of this technique in continuing pregnancies. Unlike fetoscopy, endoscopy uses a transcervical approach with a larger-diameter scope equipped with a wide-angle lens; the procedure requires no anesthesia and permits a larger field of view.

Transcervical endoscopy can be useful in the verification of suspected structural anomalies before elective termination of pregnancy.

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Trophoblast from anembryonic pregnancy has both a maternal and a paternal contribution to its genome

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In a series of 14 anembryonic pregnancies, deoxyribonucleic acid from trophoblast was examined with locus-specific minisatellite probes. It was found in each case that trophoblast from anembryonic pregnancy has both a maternal and a paternal contribution to its genome. This means that although anembryonic pregnancy shares characteristics with complete hydatidiform mole and androgenetic development in the mouse in that there is no embryo, it does not arise by the same genetic process. Of three anembryonic pregnancies that were successfully karyotyped, two had normal female 46,XX chromosome constitutions and one had an abnormal 47,XX + 16 complement. The sex of the trophoblast in each anembryonic pregnancy was determined with a deoxyribonucleic acid probe with Y-specific bands. A male-to-female ratio of 6:8 was found, which is not significantly different from normal. (AM J OBSTET GYNECOL 1991;165:98-102.)

Key words: Anembryonic pregnancy, locus-specific minisatellite deoxyribonucleic acid probes, hydatidiform mole

Anembryonic pregnancy or blighted ovum is a condition in which there is no embryo proper but there is apparently normal development of the extraembryonic tissues. As many as 16% of all clinically recognized pregnancies may be anembryonic.[1] These pregnancies are usually first seen clinically between 8 and 14 weeks' gestation as incomplete or missed abortions but are now commonly identified by ultrasonographic imaging in early pregnancy. Abnormal sonar features include loss of definition of the gestational sac, a gestational sac that is small for dates, failure of growth of the sac, and absence of both fetal echoes and fetal heart activity. A final diagnosis of anembryonic pregnancy is made when no fetal echoes can be seen and when consequent examination of the retained products of conception reveals no fetal parts.

Very little is known about anembryonic pregnancy and its causes. It may be that the embryo fails to develop beyond a very early stage, or that it develops normally for some time and is subsequently shed, leaving the remaining trophoblast viable. Anembryonic development is common in extrauterine pregnancies with rates of 40% and higher quoted in the literature.[2] Several authors consider there to be a relationship between the length of time of intratubal residence and the development of anembryonic pregnancy.[3, 4] In extrauterine pregnancies it has been suggested that anembryonic development arises predominantly as a result of exogenous influences.[5] Why development of anembryonic pregnancy is more common in extrauterine pregnancy, and whether this bears any relationship to intrauterine anembryonic pregnancy is unknown.

Studies in the mouse have revealed that the developing conceptus needs both a maternal and paternal contribution to its genome. Mouse androgenones are produced by removal of the maternal pronucleus from a zygote and its replacement with another male pronucleus. These androgenones often fail to implant. Those that do implant have developmentally retarded embryos, although the trophoblast develops relatively normally.[6] Gynogenones are produced by adding a female pronucleus to a haploid parthenogenetic egg. These generally develop poorly after implantation, although in some cases a small but apparently normal embryo is produced. However, in these cases the extraembryonic tissues are poorly developed.[7] Therefore the maternal genome appears to be largely responsible for the development of the embryo, with the paternal genome being largely responsible for the development of the extraembryonic tissues. Androgenetic development resembles anembryonic pregnancy, with relatively normal development of the extraembryonic tissues but little or no development of the embryo proper.

In human beings, complete hydatidiform mole is a type of anembryonic pregnancy. In complete mole

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there is no development of the embryo proper but there is abnormal development of the extraembryonic tissues. Complete hydatidiform mole usually has a 46,XX chromosome constitution, all of which are paternally derived.[8] The most common mechanism by which complete hydatidiform mole arises is fertilization of an anucleate ovum by a haploid sperm that then divides to give the 46,XX chromosome constitution.[8] Histologically some similarities exist between trophoblast from anembryonic pregnancy and complete hydatidiform mole including enlarged villi, cytotrophoblast proliferation, and localized molar degeneration of villi. In exceptional cases avascular chorionic villi may be seen in trophoblast from anembryonic pregnancy.[2]

In view of the similarities between human anembryonic pregnancy, complete hydatidiform mole, and mouse androgenone development, we have performed experiments to determine the parental origin of the trophoblast from anembryonic pregnancy with the use of single locus-specific minisatellite deoxyribonucleic acid (DNA) probes.

Material and methods

In this study a clinical diagnosis of anembryonic pregnancy was made when no embryo proper could be identified within a gestation sac of >4 ml at two examinations separated by at least 1 week. A total of 19 anembryonic pregnancies were identified by ultrasonography at Queen Charlotte's and Chelsea Hospital and Hammersmith Hospital, London. Approval for the study was obtained from the local ethical committee. Products of conception were obtained as soon as possible after evacuation of the uterus. A small piece of trophoblast tissue was stored at 4° C in sterile saline solution and then cultured for chromosomal studies, the rest was frozen at −70° C. A blood sample was collected from each parent into ethylenediaminetetraacetic acid tubes and stored at −70° C. DNA was made from the tissues and blood with standard techniques. DNA (4 μg) was digested for 6 hours at 37° C with the restriction enzyme *Hin*fI. The fragments were separated in a 0.8% agarose gel by electrophoresis. Southern blotting was used to transfer DNA to Hybond-N nylon filters (Amersham International, United Kingdom). Filters were prehybridized overnight at 65° C in 10 × Denhardt's solution, 6 × standard saline citrate (20 × standard saline citrate is 3 mol/L sodium chloride, 0.3 mol/L sodium citrate), 0.5% sodium dodecylsulfate, 0.005 mol/L ethylenediaminetetraacetic acid pH 8, 2% sodium pyrophosphate, and 0.1 mg/ml denatured salmon sperm and then hybridized overnight in the same solution with 10% dextran sulfate, 200 μg/ml sheared single-stranded human placental competitor DNA, and 10 ng of the locus-specific probe pλg3.[9] The probe was labeled with phosphorous 32 deoxycytidine triphosphate by random hexanucleotide priming.[10] Filters were washed to a stringency of 0.1 × standard saline citrate at 65° C and then exposed to x-ray film for 3 days at −70° C in the presence of an intensifying screen.

The probe was subsequently stripped from the filter by washing in 0.1 mol/L sodium hydroxide for 20 minutes followed by washing in 0.5 mol/L Tris hydrochloride base with 3 mol/L sodium chloride, pH 7.5 for 10 minutes at room temperature. Further hybridization was carried out with the locus-specific probes λMS31 and λMS43.[11] Probes pλg3 and λMS31 are located on chromosome 7, with λMS43 located on chromosome 12.[12] All three probes have a heterozygosity within the general population of above 93%.[11]

A variable number of tandem repeats probe from the X chromosome, S232, which has homology to the Y chromosome and therefore has Y-specific bands,[12] was used to probe *Taq* I–digested DNA from both the parents, and trophoblast from the anembryonic pregnancy. From these autoradiographs the sex of the trophoblast from each anembryonic pregnancy could be established.

Sections were taken from each trophoblast sample, stained with hemotoxylin and eosin, and examined for maternal tissue contamination.

Results

Of the 19 anembryonic pregnancies, histologic examination showed contamination by maternal decidua in five; these instances were excluded from further study. In no case did histologic examination identify fetal parts.

With use of the locus-specific minisatellite probes all 14 of the trophoblast samples from anembryonic pregnancies were shown to have both a maternal and a paternal genetic contribution to their genome. Both a maternal and a paternal band could be seen in each (Fig. 1). In one case four *Hin*fI restriction fragments were seen in the trophoblast lanes with the probe pλg3, two being derived from each parent. However, with the locus-specific probes λMS31 and λMS43 only three bands could be seen.

With the X-chromosome probe S232, which has Y-specific bands, it was found that the female-to-male sex ratio for the trophoblast from anembryonic pregnancy was 8:6, which is not significantly different from normal.

Karyotyping was successful in only three cases. Of these, two cases had a normal 46,XX chromosome complement and one was abnormal, 47,XX + 16. Karyotyping was frequently unsuccessful because of failure of the cultures to grow or infection of the cultures.

Comment

It is apparent from this study that trophoblast in anembryonic pregnancy does not arise by the same ge-

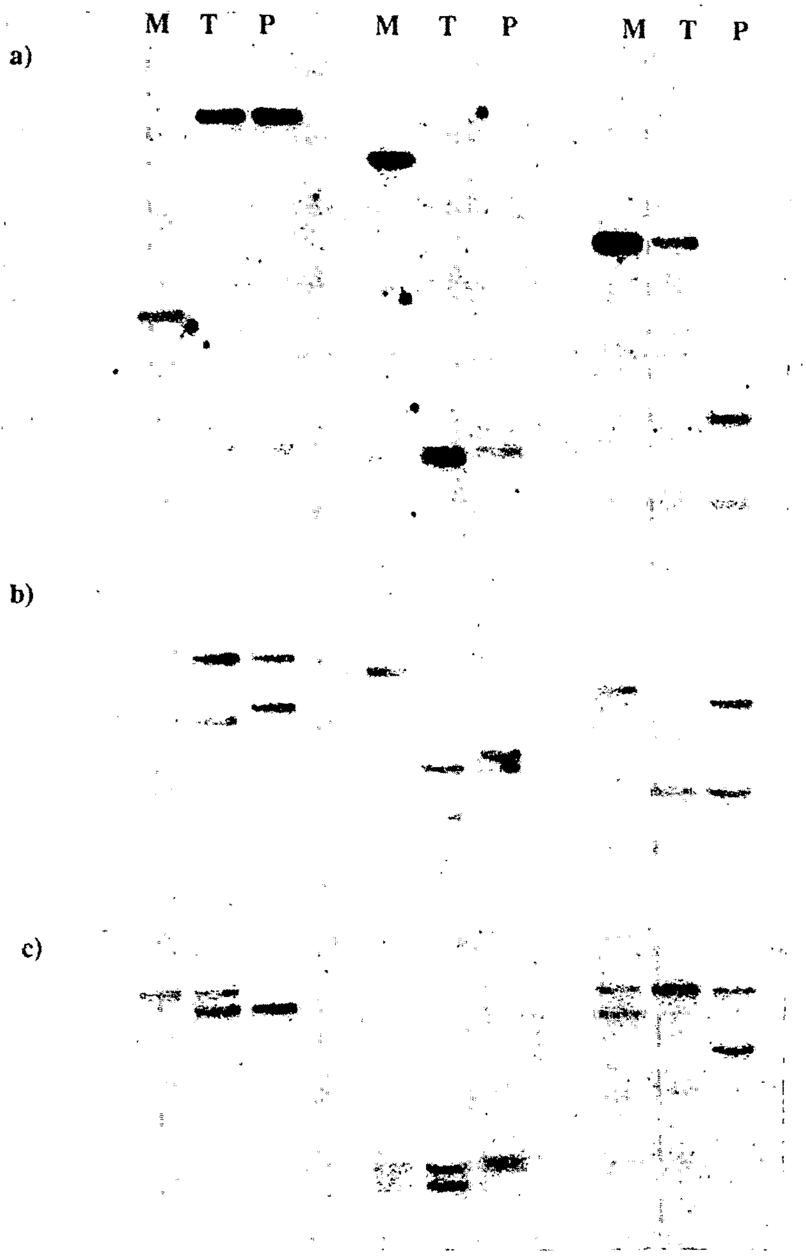

Fig. 1. Autoradiographs of three data sets of DNA from maternal blood *(M)*, trophoblast from anembryonic pregnancy *(T)*, and paternal blood *(P)* probed with three locus-specific minisatellite probes **a**, λpg3; **b**, λMS31; and **c**, λMS43.

netic mechanism as does complete hydatidiform mole, inasmuch as the tissue has both a maternal and a paternal contribution to its genome. This also suggests that anembryonic pregnancy is not equivalent to the generation of androgenones in the mouse, which have extraembryonic tissues at the implantation site but little or no development of the embryo proper. In the case in which four bands were seen in the trophoblast lane with the probe pλg3, it is likely that the tissue resulted from a twin pregnancy. With the probes λMS31 and λMS43 three bands were seen in the trophoblast lane, one band presumably being shared by the twin trophoblasts. Because there was no sharing of alleles by the parents, tetraploidy would be expected to produce four bands with each probe. Previous studies have suggested that up to 60% of anembryonic pregnancies show some chromosomal abnormality (Davidson HR, Duncan Guthrie Institute, Glasgow. Unpublished results). Our data so far have revealed one trisomy 16 karyotype, which is the most common trisomy found in tissues from spontaneous abortions.[14] Both of the other karyotypes obtained showed apparently normal female 46,XX chromosome constitutions. We have been unable to exclude the possibility that there are gross chromosomal abnormalities within the tissue. Triploidy, tetraploidy, and trisomies of various chromosomes have been reported in anembryonic trophoblast (Davidson HR. Unpublished results). Trophoblast from anembryonic pregnancy appears to be very susceptible to infection on culture. This could be because the tissue in the uterus is often necrotic at the time of evacuation of the uterus, decreasing viability with increased risk of infection. Alternatively the tissue may become infected while passing through the vagina. These problems could be solved by use of direct karyotyping (with no culture step), although this relies on there being cells in the metaphase stage of mitosis at the time of evacuation of the uterus. It would appear that there is no sex bias in the trophoblast from anembryonic pregnancy.

Several other mechanisms can be postulated that could result in anembryonic pregnancy. Uniparental disomy, a phenomenon whereby both of any one pair of homologous chromosomes are from the same parent, is one possible mechanism. Uniparental disomies have been reported in cases of cystic fibrosis in which only one parent was a cystic fibrosis carrier. On closer examination it became apparent that both chromosome 7s had been inherited from the one carrier parent.[15, 16] It is possible that uniparental disomies of other chromosomes might be lethal to the developing conceptus, but may allow the development of anembryonic pregnancy. Such a hypothesis can be tested with DNA probes for each chromosome in turn. Inasmuch as probes pλg3, λMS31, and λMS43 are located on chromosomes 7 and 12, we know that chromosomes 7 and 12 are not uniparentally disomic in any of our experimental samples.

In view of the similarity of our findings for trophoblast from anembryonic pregnancy and normal conceptions, it may be that anembryonic pregnancy starts normally but for some reason discards the amnion and embryo, retaining the chorion. Missed abortions are often seen with an underdeveloped embryo small for gestational age, and it is possible that this embryo is simply discarded in anembryonic pregnancy.[17]

Incorrect genomic imprinting might result in inappropriate developmental gene expression, which in turn may lead to a nonviable conceptus. Chromosomes appear to be imprinted in such a way that particular regions of DNA are expressed according to their parental chromosome origin.[18] At different developmental stages particular genes are expressed from one parent rather than from the other. Genomic imprinting is thought to be achieved by methylation of DNA at selected CpG sites upstream from the first exons of the gene. It could be that in anembryonic pregnancy, genes necessary for the development of the embryo are incorrectly imprinted and thus fail to be expressed in the developing conceptus. This could be studied by examination of the methylation status of various genes known to be important in development, in comparison with normal trophoblast.

It may be that there are differences in the level or site of genes known to be important in development, such as proto-oncogenes and growth factors, between anembryonic and normal trophoblast. It has been shown that several proto-oncogenes are expressed in a stage-specific and tissue-specific manner in the human placenta[19] and these could be examined within normal trophoblast and trophoblast from anembryonic pregnancy.

It would seem that with respect to parental origin, the trophoblast from anembryonic pregnancy is normal. This is of interest in the light of studies that involve androgenetic development in the mouse and of complete hydatidiform mole in human beings, which share similar characteristics. Further studies in the areas outlined in this article are being carried out in this laboratory and may help to determine the causes of anembryonic pregnancy.

We thank Cellmark Diagnostics for providing the locus-specific minisatellite probes λg3, λMS31, and λMS43. In addition we thank Dr. Pauline Yen for the donation of the probe S232.

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Hydrops fetalis in the McKusick-Kaufman syndrome: A case report

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McKusick-Kaufman syndrome is an autosomal recessive multiple malformation syndrome characterized by hydrometrocolpos and polydactyly. We report on a patient with McKusick-Kaufman syndrome and severe hydrops. This case illustrates the necessity of genetic evaluations for all patients with unexplained hydrops. (AM J OBSTET GYNECOL 1991;165:102-3.)

Key words: McKusick-Kaufman syndrome, nonimmune hydrops, hydrometrocolpos

Genetic causes of nonimmune hydrops are numerous but frequently are undiagnosed. We present the first report of which we are aware of McKusick-Kaufman syndrome, an autosomal recessive disorder, and classic nonimmune hydrops. Consider genetic disease in all cases of unexplained hydrops.

Case report

A 24-year-old gravida 2, para 1 woman was referred at 30-4/7 weeks' gestation. Ultrasonography revealed mild polyhydramnios, bilateral hydronephrosis, multicystic kidneys, abnormal bladder, and fetal hydrops with marked ascites. Her blood type was A positive; rapid plasma reagin test results were nonreactive. Amniocentesis and cordocentesis revealed a normal 46,XX fetal karyotype, negative Coombs test results, hematocrit 40.5%, albumin 1.9 gm/dl, total protein 3.4 gm/dl, normal immunoglobulin M, and a lecithin/sphingomyelin ratio of 1.6. On hospital day 8 emergency cesarean section was performed because of maternal fever and worsening hydrops.

A 2710 gm edematous female with Apgar scores of 5 and 8 at 1 and 5 minutes, respectively, and with postaxial polydactyly on three extremities was delivered. Urinalysis, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, blood urea nitrogen, and creatinine values were normal. Venous hematocrit was 38.2%, albumin 2.0 gm/dl, and total protein 3.2 gm/dl. The placenta was normal. A patent ductus arteriosus was surgically ligated after two courses of indomethacin (Indocin) failed. No other cardiac abnormality was found. By the time of the patient's discharge, serum albumin had increased to 4.5 gm/dl and total protein to 6.2 gm/dl.

A blind vaginal orifice was discovered in normal appearing external genitalia, suggesting a diagnosis of McKusick-Kaufman syndrome. Radiographic studies confirmed the presence of bilateral hydroureters, a dilated, fluid-filled uterus, and a normal bladder; no renal cysts were seen. Cystoscopy revealed a common genitourinary sinus with a vesicovaginal fistula. After

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vaginostomy and drainage, the blood urea nitrogen and creatinine values, which had risen to 26 mg/dl and 1.9 mg/dl, respectively, declined to 13 mg/dl and 0.5 mg/dl by 31 days. After discharge she had persistent ureteropelvic junction obstruction and subsequently underwent exploration and pyeloplasty on the left kidney for an aberrant lower pole vessel. However, obstruction and electrolyte imbalances recurred. At approximately 1 year of age she was lost to follow-up.

Comment

Nonimmune hydrops carries a poor prognosis, perinatal mortality in most series being greater than 50%. Whereas traditional studies suggest the vast majority of cases will have no identifiable etiologic factor, a cause or predisposing condition can be found in more than 75% of cases with full genetic and pathologic evaluations.[1] Because genetic disorders can have a significant recurrence risk in future pregnancies, and because the prognosis and management for affected persons may depend greatly on the underlying disease, evaluation for a possible genetic cause is imperative for all cases of unexplained nonimmune hydrops.

Whereas there are many published cases of McKusick-Kaufman syndrome,[2] this is the first report of which we are aware of true nonimmune hydrops in a fetus with classic McKusick-Kaufman syndrome. The hydrops was presumed to be a result of decreased serum oncotic pressure due to the hypoalbuminemia and hypoproteinemia, found both prenatally and postnatally. However, the cause of the low protein level is unknown because she had normal liver and renal function at birth. This patient also shows that in cases of hydrops, polyhydramnios can coexist with urinary tract obstruction when the obstruction is either partial or intermittent.

Hydrometrocolpos has been diagnosed antenatally in cases in which there is a large mass that extends into the fetal abdomen. In our patient the mass was seen on every ultrasonographic examination, but was interpreted as the fetal bladder because of its small and stable size. However, it was large enough to cause intermittent ureteropelvic junction obstruction.

This patient shows several interesting features: (1) an association between McKusick-Kaufman syndrome, a rare autosomal recessive disorder, and nonimmune fetal hydrops; (2) polyhydramnios associated with urinary tract obstruction; and (3) the importance of an interdisciplinary approach for cases of nonimmune hydrops. Our patient retained good renal function in spite of fetal and early neonatal obstruction. However, if the diagnosis can be recognized prenatally in other affected persons, intervention, such as intrauterine decompression of the dilated uterus and vagina, may prevent progressive renal compromise. Being alert to subtle clues, such as a persistently full fetal bladder, may aid in prenatal diagnosis.

Correct diagnosis, both prenatally and postnatally, of potentially treatable and recurrent causes of nonimmune fetal hydrops is critical for providing appropriate perinatal management and therapy and for accurate genetic counseling.

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Correlation between sexual abuse and somatization in women with somatic and nonsomatic chronic pelvic pain

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After a comprehensive clinical and psychological evaluation, 99 women with pelvic pain of at least 6 months' duration and normal findings at laparoscopy were divided into two groups, including 47 women with probable somatic causes of pain (group 1) and 52 women without identifiable somatic abnormality (group 2). Women without identifiable somatic abnormality (group 2) were younger, had higher mean somatization scores, and reported an earlier mean age at first intercourse, a higher number of total sexual partners, and a higher prevalence of sexual abuse before the age of 20. Within group 2 (nonsomatic pain) but not within group 1, mean somatization scores were significantly higher among women with a history of sexual abuse than among women with a negative history. When analyzed as risks for nonsomatic pelvic pain, the positive predictive value of both a history of sexual abuse and a high somatization score was 78% (relative risk compared with that of women with zero or one risk factor, 2.1; $p < 0.0001$). These data suggest that the psychosocial profile of women with nonsomatic pelvic pain differs from that of women with somatic pelvic pain and that previous sexual abuse is a significant predisposing risk for somatization and non-somatic chronic pelvic pain. (AM J OBSTET GYNECOL 1991;165:104-9.)

Key words: Hysterectomy, pain, sexual abuse, somatization

Chronic pelvic pain, defined as noncyclic pelvic pain of >6 months' duration, is a common gynecologic complaint, accounting for 10% of outpatient gynecologic consultations and indicating up to 25% of diagnostic laparoscopies.[1] More important, chronic pain is listed as the indication for 10% to 19% of hysterectomies in the United States; thus it is the third most frequent indication (after leiomyomas and abnormal bleeding) and accounts for approximately 78,000 hysterectomies annually.[2-4]

Studies comparing women with chronic pelvic pain to pain-free control subjects have demonstrated significant differences in rates of sexual dysfunction, depression, somatization, and abnormal psychological testing between pain patients and controls.[1, 5-8] By contrast, studies comparing the psychosocial profiles of women with presumedly somatic chronic pelvic pain (such as women with endometriosis) with those of women with nonsomatic chronic pelvic pain have not demonstrated any significant differences in these variables between groups.[6-10] An exception to this was the study of Magni et al.,[8] which documented a higher self-rating of depression in women with chronic pain and normal laparoscopic findings as compared with those with abnormal laparoscopic findings. These findings appear to suggest that chronic pain (somatic or otherwise) is the cause, rather than the effect, of the associated psychopathologic condition.

Unfortunately, these latter studies suffer from a consistent methodologic constraint, which is reliance on laparoscopy to discriminate "somatic pain" controls from "nonsomatic" pain study populations.[6-10] Several recent investigations have documented that laparoscopically invisible nongynecologic somatic abnormalities, such as irritable bowel syndrome, abdominal wall neural "trigger points," and chronic urethritis, may constitute many cases of pelvic pain with normal laparoscopic results.[11-13] Furthermore, two recent studies reported that the prevalence of classic, laparoscopically visible somatic intrapelvic findings, such as endometriosis and pelvic adhesions, was the same in patients undergoing laparoscopy because of pain and in pain-free patients undergoing laparoscopy because of infertility or tubal sterilization.[5, 14, 15] Together, these investigations fundamentally challenge both the sensitivity and the specificity of laparoscopy for the diagnosis of pelvic pain and the validity of the conclusions reported from trials that used this method.

High somatization (somatic symptoms) scores and preoccupation with physical symptoms and concerns

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have been observed in adult women with histories of sexual and nonsexual physical abuse.[16-18] Several groups, including ours, have reported higher rates of both somatization and childhood sexual trauma in women with chronic pelvic pain as compared with pain-free control subjects.[1, 5] However, the correlation between these variables within a population of women with chronic pelvic pain (or subsets of women with somatic or nonsomatic chronic pelvic pain) has not been reported.

The purpose of this study was twofold. First, we wished to compare the demographic and psychosocial profiles of groups of women with somatic and nonsomatic pelvic pain by means of diagnostic methods other than laparoscopy to distinguish groups. Second, we wished to correlate history of sexual trauma and somatization in groups of women with chronic pelvic pain with and without identifiable somatic pathology.

Material and methods

Between June 1986 and January 1989, 135 women were referred to a multidisciplinary pelvic pain clinic at San Diego Naval Hospital. Ninety-nine women who completed a comprehensive medical and psychological evaluation comprise the study population. The demographic profile of this population has been reported previously.[1] All of these women had complained of pelvic pain of at least 6 months' duration and had undergone a baseline workup consisting of history and physical examination, laboratory evaluation, and laparoscopy, the results of which were considered normal by the referring physician. The study was reviewed and approved by our institutional review board.

All patients completed a comprehensive medical and historical questionnaire conducted by private structured interview with a female gynecology nurse practitioner. In addition to standard medical and pain information, all subjects completed a detailed sexual history, including age at first coitus, current and total numbers of sexual partners, and history of previous major psychosexual trauma, defined as incest (coitus with a legal relative), repetitive sexual molestation (noncoital genital touching or exposure with or without vaginal, oral, or anal penetration), or rape before the age of 20. Isolated instances of molestation were not included in this definition for purposes of tabulation. The medical questionnaire included a standard screening protocol for irritable bowel syndrome, as described elsewhere.[11]

A somatization scale, which has been previously reported,[1] consisting of 26 common somatic and emotional symptoms unrelated to pelvic or abdominal pain was administered to all subjects (Table I). A net somatization score was determined for each patient on the basis of the total number of items endorsed as sufficient to require medical consulation or to interfere with usual daily activities. For purposes of calculating predictive values for nonsomatic pelvic pain, somatization scores of 0 to 7 were arbitrarily defined as "low" and scores from 8 to 26 were defined as "high."

Table I. Questionnaire of unrelated somatic and psychologic symptoms

Please check symptoms for which you have been treated or have seen a doctor.

A. ____ Headache
B. ____ Low back pain
C. ____ Pain with urination
D. ____ Sleeplessness
E. ____ Weight loss or gain
F. ____ Dizziness
G. ____ Passing out
H. ____ Depression
I. ____ Premenstrual syndrome
J. ____ Shortness of breath
K. ____ Joint pain
L. ____ Nausea
M. ____ Bloating
N. ____ Vomiting
O. ____ Diarrhea
P. ____ Constipation
Q. ____ Fatigue
R. ____ Loss of appetite
S. ____ Mood swings
T. ____ Stress or nervousness
U. ____ Palpitations
V. ____ Heartburn
W. ____ Vaginal discharge
X. ____ Leg pain
Y. ____ Hot flashes
Z. ____ Low sexual desire

(From Reiter RC, and Gambone JC. Demographic and historical variables in women with idiopathic chronic pelvic pain. Obstet Gynecol 1990;75:428. By permission of the American College of Obstetricians and Gynecologists.)

The standardized medical evaluation and diagnostic criteria for somatic diagnoses used in the clinic have been reported previously.[13] Briefly, all patients underwent a repeat physical examination, which included specific attempts to elicit trigonal or urethral tenderness or abdominal wall myofascial pain or "trigger points" as described by Slocumb,[12] Thompson and Francis,[19] and Applegate.[20] Screening laboratory tests included complete blood count, stool guaiac test, urinalysis and urine culture, cervical culture, cytologic testing, and chlamydial antigen testing. Additional special studies, such as bowel radiographs, urethrocystoscopy, intravenous pyelography, and abdominal trigger point injections, were obtained as indicated.

On the basis of the presence or absence of identifiable somatic abnormality, patients were divided into two groups. Group 1 consisted of 47 women with probable somatic pelvic pain and group 2 consisted of 52 women without identifiable somatic abnormality. The diagnostic profiles of patients in group 1 are summarized in Table II. All statistical comparisons were done with the χ^2 or Student t test as appropriate.

Results

Group comparisons for variables of interest are summarized in Table III. The mean ages of 47 women with somatic pelvic pain (30.0 ± 7.7 years) were significantly older than those of 52 women with nonsomatic pain (27.3 ± 6.0 years), although the duration of pain was

Table II. Diagnoses in 47 women with identifiable somatic abnormality

Diagnosis	*No.*	*%*
Abdominal wall–myofascial pain	15	32
Atypical dysmenorrhea or ovulatory pain	10	21
Irritable bowel syndrome	8	17
Urethral syndrome	3	7
Symptomatic pelvic vascular congestion	2	4
Chlamydial endometritis or salpingitis	2	4
Miscellaneous*	7	15
TOTAL	47	100

*Includes one case each of interstitial cystitis, detrusor dyssynergia, symptomatic pelvic relaxation, incarcerated ventral hernia, adenomyosis, residual accessory ovary, chronic coccygeal pain.

similar for both groups. No significant differences in cumulative gravidity or parity, racial distribution, educational attainment, marital status, or cumulative operations or current medications were observed between the two groups. However, women with nonsomatic pelvic pain reported a younger age at first intercourse (15.2 ± 3.2 vs 17.9 ± 3.0 years), a higher number of total sexual partners (6.7 ± 6.2 vs 4.6 ± 5.0), and a substantially higher prevalence of sexual abuse before the age of 20 (67% vs 28%), as compared with women with somatic diagnoses.

Somatization scores in both groups stratified by history of sexual trauma are summarized in Table IV. A strong correlation was observed between somatization score and a history of sexual trauma in group 2 (nonsomatic pain), with a mean somatization score that was nearly twice as large for women reporting a history of sexual trauma (10.3 ± 4.8 vs 5.9 ± 2.9). However, no similar correlation was observed in group 1 (somatic pelvic pain).

To calculate the predictive value of history of sexual abuse and high somatization score for nonsomatic pelvic pain, cases were identified in which both of these variables were present. In the entire study population there were 37 patients who were considered to have a "high-risk" profile, defined as both a history of sexual abuse and "high" somatization scores (≥ 8). Somatic diagnoses were rendered in only eight (22%) of these women, yielding a positive predictive value of both risk factors for nonsomatic pelvic pain of 78%. Conversely, there were 62 subjects who were considered to have a "low-risk" profile (zero or one risk), defined as either a history of sexual abuse or a high somatization score (≥ 8) or neither of these. Somatic diagnoses were established in 39 of these women, yielding a negative predictive value for zero or one risk factor for nonsomatic pelvic pain of 63% ($p < 0.0001$) (cumulative relative risk for nonsomatic pelvic pain for women with both risks as compared with women with zero or one risk factor, 2.1).

Comment

Chronic pelvic pain, the third most common indication for hysterectomy, which is the second most frequent major surgical procedure in the United States, is a significant health concern of women. Histologic abnormality (usually an incidental leiomyoma) was discovered in fewer than half (41%) of the hysterectomy specimens in cases of chronic pain analyzed in a large collaborative review from the United States.[4] This symptom may indicate the presence of gynecologic disease, such as recurrent salpingitis; nongynecologic somatic abnormalities, such as abdominal trigger points; or nonsomatic (psychological) pathology, such as somatization disorder. Although the majority of these patients present to gynecologists for evaluation and treatment, the majority do not have gynecologic pathology.

Traditionally, the gold standard of gynecologic evaluation of chronic pelvic pain has been laparoscopy. A common tendency has been to assume that any visible intrapelvic abnormality, such as endometriosis or pelvic adhesions, is causally associated with the pain and that causes have been excluded if the laparoscopic findings are normal. However, there are no objective data to support these assumptions, and a substantial body of controlled investigation suggests that they are invalid.

Rapkin[14] has recently investigated the association between pelvic adhesions and chronic pelvic pain. In a review of 188 laparoscopies Rapkin[14] observed no difference in the prevalence of pelvic adhesions in patients with chronic pain as compared with pain-free infertile control subjects. Furthermore, she was unable to correlate the location or severity of pain with the location or severity of adhesions observed at laparoscopy. Similarly, the correlation between the observed severity of endometriosis (as determined by various classification schemes) and associated chronic pain is notoriously poor, with the majority of patients with mild, moderate, and severe disease reporting no symptoms at all.[21] Vernon et al.[22] have recently demonstrated in tissue culture that morphologically and visually "classic" (powder burn and mulberry spot) endometriotic implants represent inert hemosiderin "tattoos" and that metabolically active (prostaglandin-producing) endometriosis is present in morphologically subtle pink or tan implants. In a recent prospective study, Walker et al.[5] observed no difference in the prevalence of endometriosis or pelvic adhesions in groups of patients with pelvic pain and pain-free control subjects who were undergoing laparoscopy for tubal sterilization or infertility.

While these investigations have questioned whether "classic" gynecologic findings, such as endometriosis and adhesions, actually cause chronic pain, other investigations have questioned the ability of laparoscopy to rule out many somatic causes of chronic pelvic pain. Slocumb[12] diagnosed abdominal wall "trigger

Table III. Sexual histories and somatization scores

	Somatic pelvic pain	*Nonsomatic pelvic pain*	*p Value*
No.	47	52	
Mean age (yr)	30.0 ± 7.7	27.3 ± 6.0	<0.05
Duration of pain (yr)	2.4 ± 2.1	2.7 ± 2.2	NS
Age at first coitus (yr)	17.9 ± 3.0	15.2 ± 3.2	<0.001
Total No. of sexual partners*	4.6 ± 5.0	6.7 ± 6.2	<0.05
No. with sexual abuse or trauma†	13 (28%)	35 (67%)	<0.001
Somatization score	6.8 ± 4.0	8.9 ± 4.8	<0.02

*Excluding sexual abuse or nonconsensual partners.
†Incest, repetitive molestation, or rape before age 20.

points" in 131 (74%) of 177 patients referred for evaluation of chronic pelvic pain. Similarly, Hogston[11] reported that symptoms consistent with irritable bowel syndrome were evident in >60% of gynecologic referrals for chronic pelvic pain. Although the high prevalences reported for these diagnoses probably reflect observer biases, these and similar studies confirm that laparoscopy is an inadequate means of ruling out many somatic causes of pelvic pain. Our experience has confirmed this impression. We recently reported the results of clinical evaluation of >200 patients referred for evaluation of pelvic pain after normal laparoscopic findings.[13] Laparoscopically occult somatic pathology was diagnosed in almost half of these patients, with gynecologic diagnoses (usually atypical dysmenorrhea) accounting for <20%.

The implications of these data for previous investigations regarding the psychological profile of women with chronic pelvic pain are profound. Studies by Walker et al.,[5] Beard et al.,[6] Renaer et al.,[7] and Magni et al.[8] documented significantly higher rates of depression, somatization, substance abuse, adult sexual dysfunction, and "neurosis" in women with chronic pelvic pain as compared with pain-free control subjects. However, such investigations inherently fail to control for the confounding variable of chronic pain (somatic, pelvic, or otherwise), which has been shown to predispose to many of these disorders.[23-26] By contrast, studies by Beard et al.,[6] Renaer et al.,[7] Rosenthal et al.[9] and Magni et al,[8, 10] which compared patients with presumed nonsomatic pain with somatic pain control subjects, demonstrated no significant differences in rates of abnormal psychological testing, somatization, or sexual dysfunction. However, without exception, these studies relied on laparoscopy to distinguish study and control populations. In view of the investigations cited above, it seems probable that some patients with undiagnosed somatic abnormalities were included in the study populations with normal laparoscopic findings and that the presumedly somatic causes of pain in the control group with abnormal laparoscopic findings (almost uniformly pelvic adhesions or endometriosis) were in fact frequently spurious.

Table IV. Somatization scores and history of sexual trauma

	Sexual abuse history		
	*Positive**	*Negative*	*p Value*
TOTAL ($N = 99$)			
No.	48	51	
Symptom score	9.9 ± 4.8	6.1 ± 3.4	<0.001
Somatic pain (group 1; $n = 47$)			
No.	13	34	
Symptom score	8.6 ± 4.4	6.2 ± 3.6	NS
Nonsomatic pain (group 2; $n = 52$)			
No.	35	17	
Symptom score	10.3 ± 4.8	5.9 ± 2.9	<0.002

*Incest, repetitive molestation, or rape before age 20.

In this study all patients were referred after normal laparoscopic results, thereby eliminating inclusion of spurious laparoscopic findings in either group. Furthermore, a specific and systematic attempt was made to diagnose nongynecologic somatic causes of pain with the use of recently reported criteria and methods. What emerged from this approach was a distinct psychosocial profile of patients with and without identifiable somatic pathology. Although mean duration and severity of pain were similar, women with nonsomatic pain were younger and had significantly higher mean somatization scores. The most striking population differences involved sexual history, with patients with nonsomatic pelvic pain reporting significantly younger mean ages at first intercourse, higher total numbers of sexual partners, and higher prevalence of significant, usually childhood and adolescent sexual abuse.

Childhood sexual abuse has been associated with numerous sequelae in adult women, including sexual dysfunction, promiscuity, dependency, depression, and somatization.[18, 27-32] We observed an association between somatization and history of sexual trauma in women with nonsomatic pelvic pain. Although this relationship was not observed in women with somatic pelvic pain, the mean somatization score among women with somatic diagnoses and a history of sexual abuse (8.6) was higher than that among women with somatic diagnoses with a negative history of abuse (6.2). That this difference did not attain statistical significance may reflect either the small sample size ($n = 13$) in the former or simply selection of a population of women in whom painful somatic pathology is incidentally, rather than causally, associated with a history of sexual abuse.

These findings provide strong presumptive evidence that nonsomatic pelvic pain is one manifestation of somatization associated with sexual trauma. They do not address the more fundamental question of whether sexual abuse causes pelvic pain or is simply part of a larger pattern of childhood and adolescent abuse, neglect, and chaos. One possible explanation for these findings would be that women who somatize are more likely to exaggerate or fabricate histories of sexual trauma. However, Morrison[32] concluded that there was no objective basis for this assertion and that histories were characteristically consistent, detailed, and verifiable. Whether there is any specificity to the association of genital trauma (as opposed to nonsexual physical abuse) and chronic genital (as opposed to nongenital) pain is a logical and attractive hypothesis that remains speculative at this time. For example, Rapkin et al.[33] has recently reported a higher prevalence of childhood physical, but not sexual, abuse among women with chronic pelvic pain as compared with nonpelvic pain and pain-free control subjects. Although the explanation for the low prevalence of sexual abuse in her series is not clear, her results do emphasize that abuse, whether physical or sexual, may predispose to chronic pain.

A second question posed by this study is whether some of the somatic abnormalities observed in group 1 may actually represent symptoms of underlying psychological disorders rather than primary diagnoses. This is a reasonable objection since high rates of hypochondriasis and obsessive neurosis have been reported in women with conditions such as irritable bowel syndrome.[34] These considerations and the psychological impact of chronic pain undoubtedly help to explain the high mean somatization scores (6.8) in the somatic pain group as compared with previously reported pain-free control subjects (2.0).[1] Nevertheless, the fact that women with somatic diagnoses had a distinct psychosocial and sexual profile at least suggests that the cause and psychogenesis of the psychologic abnormality represented in this group may be different from those associated with nonsomatic pain.

A history of sexual trauma and a high somatization score were found to be predictive of increased risk for nonsomatic pelvic pain. The practical significance of this observation is readily appreciated since it should serve to alert the clinician to patients who should be referred for psychologic evaluation and treatment. We are currently in the process of prospectively validating a simple self-reporting questionnaire that may help in assessment of risk for nonsomatic pelvic pain and psychogenic pain disorders (unpublished data). Meanwhile, it seems clear that hysterectomy is not indicated for treatment of chronic pelvic pain before thorough medical and psychologic evaluation and should be performed only when gross anatomic uterine pathology (such as procidentia or large leiomyomas) that is sufficient to explain the degree of disability has been documented.

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Cesarean section by local anesthesia in patients with familial dysautonomia

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We describe a 29-year-old patient with familial dysautonomia who underwent cesarean section because of severe intrauterine fetal growth retardation. The surgery was done after induction of local anesthesia to avoid the critical and sometimes fatal complications of general anesthesia known in patients with familial dysautonomia. Surgery was uneventful and almost painless. The postoperative period was without complications. Induction of local anesthesia for cesarean section may constitute a suitable alternative in patients with familial dysautonomia. (AM J OBSTET GYNECOL 1991;165:110-1.)

Key words: Familial dysautonomia, Riley-Day syndrome, anesthesia

Familial dysautonomia or Riley-Day syndrome is a rare, autosomal recessive genetic disorder found in the Ashkenazi Jewish population that affects the development of the autonomic and sensory nervous systems. Characteristic signs and symptoms include profuse sweating, vomiting, lack of overflow tears, lung infections after frequent episodes of aspirative pneumonia, scoliosis, cardiovascular instability, impaired heat perception and taste, and decreased sensation of pain.[1]

In the past few patients survived long enough to become pregnant; therefore there is little experience with delivery reported in the medical literature.[1, 2] Anesthesia for surgical delivery in these patients presents particular problems. Increased morbidity and mortality were found in subjects with familial dysautonomia who required induction of general anesthesia, and dehydration, dysautonomic crisis, intraoperative severe hypotension, and postoperative atelectasis were frequently described.[2] Extradural anesthesia or spinal block are not adequate for the blood pressure lability. We present a patient with familial dysautonomia who was abdominally delivered of an infant after induction of local anesthesia to avoid the risks of the other anesthetic techniques.

Case report

A 29-year-old Jewish nulliparous patient with familial dysautonomia was first seen at 8 weeks' gestation. She worked as a volunteer in one of the hospital offices. Her previous history and signs revealed convulsive disorders during the school years, growth retardation, vomiting, episodes of lung infection, lack of fungiform papillae on the tongue, and dry eyes, all of which indicated mild familial dysautonomia. Three years before pregnancy a chest roentgenogram was normal. The final diagnosis of familial dysautonomia was made by a pathologic histamine test. The patient was pale; height was 145 cm and weight was 40 kg. She had speech difficulty, infrequent heavy coughing, and postural hypotension. Blood pressure measurements were 150/90 mm Hg while supine and 75/40 mm Hg while standing. Previous gynecologic anamnesis was unremarkable. Menses started at the age of 12 years and were regular (4 to 5 days every 30 days). Vomiting during pregnancy was infrequent. Successive control visits revealed a normally developed pregnancy. Hemoglobin level was 11 gm/dl, and the patient took multivitamin and mineral capsules. Amniocentesis was done at 16 weeks' gestation and revealed normal fetal karyotype and amniotic fluid level of α-fetoprotein.

Several episodes of hypotension occurred at home on hot days and prompted hospitalization. Biochemical tests and arterial blood gas values were in the normal range. Simple hydration was enough to restore blood pressure and a well feeling. At 34 weeks' gestational age, fetal growth retardation was established sonographically. Fetal weight was calculated as 1520 gm (less than the 10th percentile). Nonstress test revealed a normal fetal heart rate. At 36 weeks' gestation severe fetal deterioration was diagnosed and we decided to terminate the pregnancy abdominally. In light of the serious risk of induction of general anesthesia in these patients, cesarean section after induction of local anesthesia was suggested. A team of anesthetists was ready at the surgical theater to induce immediate general anesthesia if required.

Local anesthesia was induced by 1% lidocaine hydrochloride solution. Subcutaneous infiltration was

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made at both sides of a longitudinal infraumbilical incision. Subsequently, every layer was infiltrated. The abdominal wall was opened almost without any complaints by the patient. The approach to the uterus was slightly painful. The anesthetic agent was injected into the myometrium before it was incised transversally in the low segment. At this time the patient's talk showed confusion for a few minutes. A living female infant was delivered through the placenta with Apgar scores of 8 at 5 minutes and 10 at 10 minutes. The infant birth weight was 1630 gm, and results of the physical examination were normal. After extraction of the placenta, suture of the uterine wall required administration of additional local anesthetic agent. Closure of the abdominal wall was performed as usual. For the skin suture lidocaine was added. At the end of the operation the patient was happy and content. The postoperative period was simple and uneventful.

Comment

Familial dysautonomia (Riley-Day syndrome) is a rare disease. The experience with pregnant patients is sparse and only two deliveries by cesarean section were reported in the literature,[2] both of which were performed after induction of general anesthesia. Familial dysautonomia constitutes an anesthetic hazard and increased morbidity and mortality were reported. Cardiovascular lability, respiratory depression, and atelectasis, are grave complications described in surgery with induction of general anesthesia. Adequate hydration and careful titration of anesthetic agents were recently recommended to minimize intraoperative and postoperative complications.[2] Although attractive, neither extradural nor spinal block is the prefered method to induce anesthesia in patients with dysautonomia because of cardiovascular instability.

In our patient the difficulties and risks of induction of general anesthesia were obviated by induction of local anesthesia. Induction of local anesthesia by infiltration is a useful technique that may be indicated in high-risk patients. Cesarean section was performed without trouble with lidocaine 1%, a rather high concentration, as a local anesthetic agent. Although lidocaine by intravenous route is commonly used at the dose of 1 to 5 mg/kg, higher doses may be considered when other means of administration are used. The main adverse effects of lidocaine are on the central nervous system, and it may be that the slight confusion of the patient during surgery was a result of the anesthetic agent. Because a large quantity of anesthetic solution is needed, it may be recommended in future cases to use lidocaine solution 0.5% with epinephrine to slow absorption and reduce untoward effects.

Because of greater understanding of the disease and better treatment today, more patients with familial dysautonomia reach adulthood and can get pregnant. It is our conclusion that when cesarean section is required, induction of local anesthesia may constitute a prefered alternative in these patients.

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Tumor necrosis factor-α and interleukin-1β production by human fetal Kupffer cells

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This study describes the isolation and characterization of human fetal Kupffer cells. We demonstrated that these cells have the potential to respond to cytokines and lipopolysaccharide with an increased production of tumor necrosis factor-α and interleukin-1β. Kupffer cells were characterized by: (1) morphologic characteristics after adherence to plastic, (2) staining for α-naphthyl acetate esterase, (3) immunofluorescence with monoclonal antibodies, and (4) phagocytosis of latex beads. More than 90% of the adherent cells were identified as macrophages. Kupffer cells cultured with lipopolysaccharide were able to produce interleukin-1β and tumor necrosis factor-α in a time- and dose-dependent fashion and maximal secretion was observed with the use of 10 μg of lipopolysaccharide per milliliter within 8 hours of treatment. We have demonstrated mature functional activity of human fetal Kupffer cells at an early gestational age (13 to 19 weeks) and discussed the roles that these cells may play in development and protection of the fetus. (AM J OBSTET GYNECOL 1991;165:112-20.)

Key words: Kupffer cells, interleukin-1β, tumor necrosis factor-α, human fetus

Kupffer cells, which represent the largest population of resident tissue macrophages in the adult,[1] play major roles in antigen processing and elimination, detoxification of endotoxin, secretion of cytokines, mediation of multiple immune reactions, maintenance of hepatocyte stability and function, and lipid and cholesterol homeostasis.[2, 3] In humans the fetal liver, as a fraction of body mass, is large compared with that of the adult. The liver represents approximately 10% of the total fetal weight at 10 weeks' gestation.[4] The human fetal liver has a diverse cellular composition with about 60% parenchymal cells (hepatocytes), 20% nonparenchymal cells (including Kupffer cells and fat storing cells), and 20% hematopoietic cells.[5]

Although the presence of Kupffer cells at early stages of human development is well documented,[6-10] the functional capabilities of these cells have not been clearly determined. Kupffer cells have been identified in sinusoid-like structures at 5 weeks' gestation and were present in fully developed sinusoids between 6 to 8 weeks.[6, 7] Macrophage precursors, originating from the yolk sac, have been identified in fetal liver at 9 to 10 weeks postconception.[8] It has been suggested that these cells function in removal of debris as one maturing embryonic tissue replaces another.[9] Indeed, yolk sac macrophages from 7-week gestations were noted to contain primitive erythroblasts.[10]

In the adult, Kupffer cell functions are well defined, and they play important roles in the clearance of biologically harmful materials, including bacterial lipopolysaccharide, microorganisms, and clotting factors.[11] Furthermore, endotoxin, derived from enteric bacterial flora, is a normal component of portal venous blood but is efficiently removed during passage through the liver by Kupffer cells.[3, 11] In addition, it is clear that Kupffer cells secrete a wide variety of cytokines that may modify immune functions, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), hepatocyte stimulating factor, and interferons.[12-15]

Important roles for fetal Kupffer cells in immune homeostasis have been proposed. Production of the important immunoregulary peptide TNF-α can be induced with endotoxin in fetal and newborn rat hepatic macrophages, demonstrating functional activity of fetal rodent Kupffer cells.[16] TNF-α exhibits diverse activities that can range from local mediation of cellular or tissue homeostasis to systemic events resulting in alterations of physiologic processes. These diverse activities depend on the concentration of TNF-α at the cellular, tissue, or systemic level.[17] Isolated monocytes from cord blood of preterm neonates produced significantly less TNF-α when stimulated with lipopolysaccharide than

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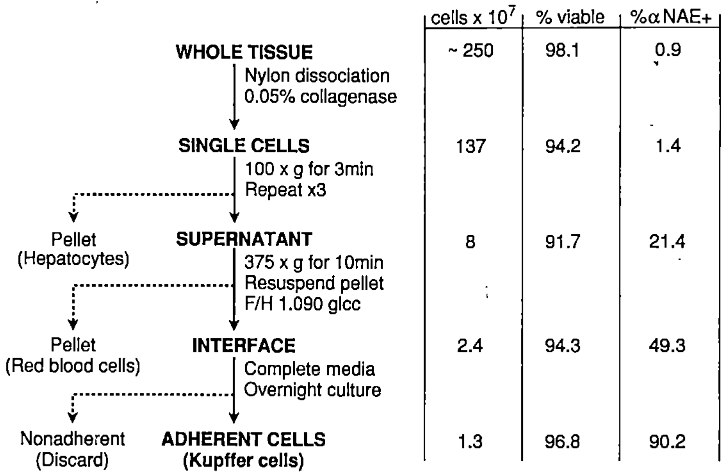

Fig. 1. Kupffer cell isolation procedure. Kupffer cells were purified from specimens of whole human fetal liver as outlined in text. Mechanical and enzymatic dissociation into single cell suspensions, followed by several centrifugation steps, preceded placing cells into overnight culture. Columns refer to total cells in a representative preparation, their viability as determined by trypan blue dye exclusion, and percentage of cells that were positive with α-naphthyl acetate esterase *(NAE)* staining. See Material and methods for details.

monocytes from term neonates, suggesting that this decreased responsiveness may contribute to the increased susceptibility of the preterm neonate to infection.[18] However, in pregnant women with preterm intraamniotic infection, increased levels of TNF-α in amniotic fluids may result in preterm rupture of the membranes.[19, 20]

We hypothesized that Kupffer cells that are obtained from fetuses at 13 to 19 weeks' gestation might possess functional activities that could be important in the development and protection of the fetus. The purposes of this investigation were (1) to develop isolation and purification techniques, (2) to establish short-term culture, (3) to characterize the functional activities, and (4) to investigate potential activators of human fetal Kupffer cells. Purified Kupffer cells could be stimulated to produce IL-1β and TNF-α. We describe a number of activators of fetal Kupffer cells and discuss the potential local (paracrine and autocrine) and systemic (endocrine) effects of these secreted mediators.

Material and methods

Human fetal tissues. Fetal tissues were obtained at the time of therapeutic termination at 13 to 19 weeks' gestation, as determined by fetal foot length. Tissues were obtained in accordance with the Donors Anatomical Gift Act of the state of Texas. A consent form and protocol approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas were used.

Kupffer cell isolation (Fig. 1). Methods used for the isolation of Kupffer cells from human fetal liver were based on published principles.[21, 22] Fresh tissue was rinsed in calcium-free phosphate-buffered saline solution, pH 7.4, with penicillin (0.1 mg/ml), streptomycin (100 μm/ml), and amphotericin (2.5 μg/ml). The liver was puncture perfused in calcium-free buffer to disrupt the desmosomal attachments of parenchymal cells,[23] then minced into 1 cm^3 pieces in 25 mmol/L HEPES with 5 mmol/L calcium buffer (pH 7.5) containing 0.05% collagenase type IV (Sigma, St. Louis) for 10 minutes at 25° C. The pieces were dissociated between layers of nylon mesh in RPMI 1640 (Gibco, Grand Island, N.Y.) with 2% heat-inactivated calf serum (Gibco), filtered through nylon, and centrifuged at 100 *g* for 3 minutes. (This pellet, depleted of Kupffer cells and cell fragments, was used as the source of hepatocytes, as described below). The supernatant, which was enriched for Kupffer cells, was centrifuged at 375 *g* for 10 minutes; then the pellet was resuspended in media, layered onto Ficoll Hypaque 1.090 gm/ml (Whitaker Bioproducts, Walkersville, Md.), and centrifuged at 350 *g* for 40 minutes at 20° C. The buffy interface was removed, rinsed three times, resuspended in RPMI 1640 with 10% fetal calf serum and antibiotics, and then placed into culture plates as described below. Cytocentrifuge preparations of various cell fractions were stained for α-naphthyl acetate esterase (Sigma), one of the most reliable markers for identification of macrophages.[11]

Hepatocytes. The pellet containing hepatocytes was treated with 0.5 mmol/L ammonium chloride buffer at 0° C for 5 minutes to lyse red blood cells. Hepatocytes were rinsed three times, then resuspended into equal volumes of Dulbecco's modified Eagle medium and Ham's nutrient mixture F-12 with 2 mmol/L L-glutamine (DME/F12, Gibco), 5% calf serum, and antibiotics. An enzyme-linked immunosorbent assay for α-fetoprotein (AFP), a product of fetal hepatocytes, was performed on cell supernatants with commercially obtained assay kits (Pharmacia, Piscataway, N.J.). Interassay coefficient of variation was <2.0%, while interassay variation was <4.8%.

Tissue culture. Kupffer cells were plated at a concentration of 2 × 10^6 cells per well onto 24-well tissue culture plates (Costar, Cambridge, Mass.) and incubated overnight at 37° C in 95% air and 5% carbon dioxide. The following day nonadherent cells were aspirated, wells were rinsed, and then 1.0 ml of complete medium was added to each well. In preliminary experiments the remaining adherent cells were counted after trypsin detachment and numbered as 1 × 10^6 cells. In certain experiments lipopolysaccharide from *Escherichia coli* 026:B6 (Sigma) was added at 0.5 to 10 μg/ml. Each experimental point was performed in triplicate, assayed in duplicate, and repeated at least five times. To determine the phagocytic capacity of the Kupffer cells, latex polystyrene beads, 0.8 μm (Sigma), or Fluorobrite latex beads, 1.75 μm (Polysciences, Warrington, Pa.), were added to adherent cells and incubated for an additional 30 minutes under the conditions described above. Cells were considered positive if ≥10 latex beads were localized intracytoplasmically by direct microscopy. Hepatocytes were plated at a concentration of 4 × 10^6 cells per well onto 24-well tissue-cultured plates (Costar) and incubated overnight at 37° C in 95% air and 5% carbon dioxide in DME/F12 medium with 5% calf serum and antibiotics. Nonadherent cells (approximately 75%) were aspirated, and then adherent cells washed and 1.0 ml of complete DME/F12 medium was added to each well before experiments were initiated. All media, calf sera, and solutions used in these experiments were tested for the presence of bacterial lipopolysaccharide as determined by the *Limulus* amebocyte lysate assay (Sigma) with a modification capable of detecting picograms of endotoxin contamination.[24] We were unable to detect endotoxin with this assay in any of our media, fetal calf sera at 20%, or other solutions used in these studies.

Other materials. Cell viability was determined by the ability to exclude 0.5% trypan blue (Flow Laboratories, McLean, Va.). Recombinant human interferon gamma 2.9 × 10^7 viral inhibition units per milligram, human insulin-like growth factor I, and rat insulin-like growth factor II were obtained from Collaborative Research (Bedford, Mass.). The following human recombinant interleukins and their stated activity were obtained from Research and Diagnostics Systems, Minneapolis: IL-1α (≥5 × 10^8 units/mg in the lymphocyte-activating factor assay); IL-1β (≥5 × 10^8 units/mg in the lymphocyte-activating factor assay); IL-3 (median effective dose of 0.5 to 1.0 ng/ml in the AML-193 incorporation assay); IL-6 (median effective dose of 0.5 to 3.0 ng/ml in the T1165.85.2.1 incorporation assay); TNF-α (≥2 × 10^7 units/mg in the L929 cytotoxicity assay); and TNF-β (>2 × 10^7 units/mg in the L929 cytotoxicity assay). Endotoxin level was determined to be <0.2 ng per 1.0 μg of cytokine by the manufacturer. Recombinant human epidermal growth factor and platelet-derived growth factor were obtained from Imcera Bioproducts, Inc. (Terre Haute).

Immunofluorescence. Monoclonal antibodies against human macrophage or monocyte cells were as follows: EMB-11 (Dakopatts, Glostrup); 3C10 (American Type Culture Collection, Rockville, Md.); anti-Leu-M5 (CD11c) and anti-Leu-M1 (CD15) (Becton-Dickinson, Mountain View, Calif.). Monoclonal antibodies against high-density determinants on human T cells were the following: anti-Leu-2a (CD8), anti-Leu-3a plus anti-Leu-3b (CD4), and anti-Leu-4 (CD3) plus anti-Leu-5b (CD2) (Becton-Dickinson). Cells were incubated with the primary antibody for 30 minutes, washed, and then incubated with fluorescein-labeled F(ab')$_2$ fragment of antimouse IgG (heavy and light chain) adsorbed with human, bovine, and horse immunoglobulin (American Qualex International, La Marata, Calif.). Cells prepared on a Cytotech centrifuge (Miles, Elkhart, Ind.) or cultured on microscope slide chambers (Flaskette, Miles Laboratories, Inc., Naperville, Ill.) were examined with a Leitz-Orthoplane microscope (Rockleigh, N.J.) with a super-high-pressure mercury lamp. Purified mouse immunoglobulin (Becton-Dickinson) was used as a negative control, with indirect immunofluorescence on fetal Kupffer cells and on Ficoll-Paque–purified mononuclear cells from adult peripheral blood. Each monoclonal reagent was tested at various dilutions and then incubated with peripheral blood mononuclear cells, as described, to determine ideal conditions and to serve as a positive control.

Assays for TNF-α and IL-1β. Two well-characterized products of macrophages include TNF-α and IL-1β. Commercially obtained enzyme-linked immunosorbent assay kits for IL-1β and TNF-α (Research and Diagnostic Systems, Minneapolis) with sensitivities of 5 pg/ml were used to assay Kupffer cell supernatants. Intraassay coefficients of variation were TNF-α, <8.0%; IL-1β, <3.0%. Interassay coefficients of variation were: TNF-α, <8.0%; IL-1β, <6.0%. In addition, TNF-α bioactivity was confirmed in certain experiments using an 18-hour cytotoxicity assay on the mouse fibroblast cell line L929 (American Type Culture Collection) as described in detail previously.[19] Recombinant human sequence TNF-α (Research and Diagnostic Systems) was used to standardize measurement of TNF-α activ-

Fig. 2. Identification of fetal Kupffer cells. **a,** Kupffer cells were incubated with 0.8 μm latex beads, washed, and then photographed to demonstrate phagocytosis. Notice numerous ingested beads *(arrow).* **b,** Kupffer cells purified up to final step before placing into culture were stained with α-naphthyl acetate. Approximately 50% of the cells demonstrated typical diffuse dark cytoplasmic staining indicating presence of nonspecific esterase *(arrow).* **c,** Kupffer cells that were adherent to culture slides were visualized by immunofluorescence microscopy after treatment with mouse monoclonal antibody 3C10 and fluorescein-labeled $F(ab')_2$ antimouse immunoglobulin G. Approximately 90% of adherent cells demonstrated bright immunofluorescence.

ity. Specific monoclonal antibody to TNF-α (Chiron Corp., Emeryville, Calif.) was able to completely neutralize the cytolytic activity attributed to TNF-α.

Results

Purification of human fetal Kupffer cells. Fig. 1 outlines the purification scheme for human fetal Kupffer cells. The final preparations were >95% viable as assessed by trypan blue exclusion, and >90% were judged to be macrophages on the basis of the ability to ingest 0.8 μm latex beads, α-naphthyl acetate esterase staining, and immunofluorescence with monoclonal antibody 3C10 (Fig. 2, *a, b,* and *c*). Fig. 2, *a,* demonstrates an adherent Kupffer cell after incubation with 0.8 μm latex beads and extensive washing to remove noningested beads. Phagocytosis of multiple beads in the cytoplasm was characteristic of adherent cells. Fig. 2, *b,* is representative of α-naphthyl acetate used as substrate to reveal the dark-stained cytoplasm identifying esterase activity in Kupffer cells. This photomicrograph is from a population of cells just before placement into culture, where typically 50% of the cells were positive. Fig. 2, *c,* is a photomicrograph of adherent Kupffer cells visualized with indirect immunofluorescence staining techniques with mouse monoclonal antibody 3C10. Typically, >90% of adherent cells were positive with this method. Similar immunofluorescence staining patterns were obtained with the use of mouse monoclonal antibodies EMB-11 and anti-Leu-M5. Less than 1% of the adherent cells were positive when stained with monoclonal antibodies anti-Leu-2a, anti-Leu-3a + 3b, or anti-Leu-4 + 5b, indicating that our populations contained very few human T cells (data not shown).

Effect of lipopolysaccharide treatment on TNF-α, IL-1β, and AFP production by human fetal liver cells. TNF-α, IL-1β, and AFP were quantified in the culture media of 1 × 10^6 adherent human fetal Kupffer cells incubated with 10 μg lipopolysaccharide per milliliter of complete culture media for 24 hours. In addition, we assayed the same products in the culture supernatants of purified human fetal hepatocytes. As shown in Fig. 3, IL-1β and TNF-α were produced by human fetal Kupffer cells in significant quantities after stimulation with lipopolysaccharide. Baseline, unstimulated production was 20 pg/ml/10^6 cells for IL-1β and 30 pg/ml/10^6 cells for TNF-α. Hepatocyte culture supernatants contained very low levels of TNF-α or IL-1β with and without lipopolysaccharide stimulation, indicating minimal contamination with Kupffer cells. As shown in Fig. 3, <30 IU AFP per milliliter per 10^6 cells was detected in culture supernatants of Kupffer cells both with and without lipopolysaccharide, indicating minimal contamination with hepatocytes. Conversely, approximately 1400 IU AFP per milliliter per 10^6 cells could be detected in supernatants from fetal hepatocytes. Lipopolysaccharide treatment had no effect on the production of AFP by hepatocytes.

Time course of IL-1β and TNF-α production by lipopolysaccharide-treated Kupffer cells. To determine the kinetics of the response to lipopolysaccharide, Kupffer cells were incubated for 2 to 48 hours with 10 μg/ml of lipopolysaccharide. Quantities of IL-1β and TNF-α released into culture supernatants were measured by enzyme-linked immunosorbent assay (Fig. 4). Increased production of IL-1β and TNF-α were detected as early as 2 hours after stimulation, and maximal production was achieved by 6 to 8 hours. Points represent the accumulation of IL-1β or TNF-α in the culture medium supernatant up to the indicated times. These data indicate that >90% of the equilibrium concentrations of TNF-α and IL-1β produced by Kupffer cells in vitro were reached in the culture supernatants after 8 hours.

Dose response of IL-1β and TNF-α production by lipopolysaccharide-treated Kupffer cells. We then determined the dose of lipopolysaccharide required to ac-

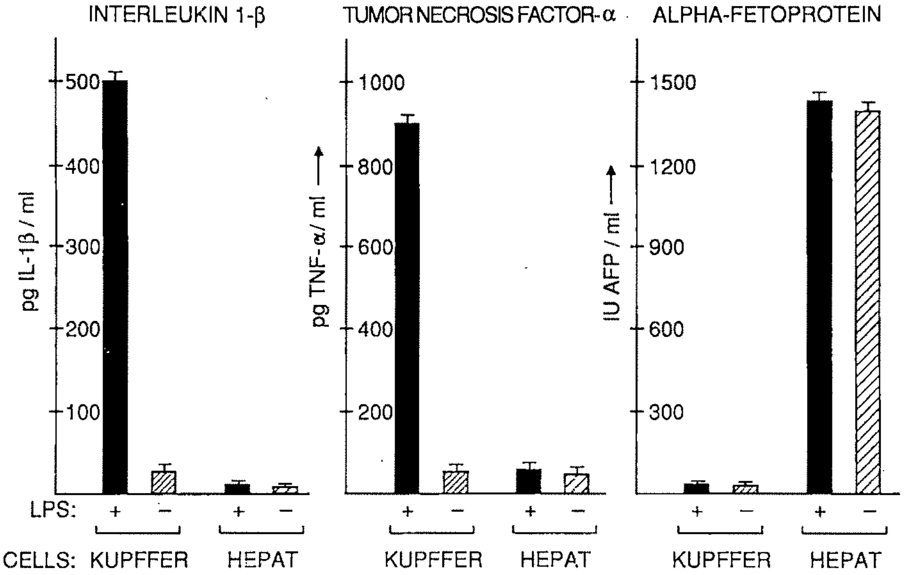

Fig. 3. Production of IL-1β, TNF-α, and AFP by cultured human fetal Kupffer cells and hepatocytes with *(solid bar)* and without *(striped bar)* lipopolysaccharide. Supernatants from overnight cultures of Kupffer cells or hepatocytes (1 × 10^6 cells) were assayed for presence of IL-1β, TNF-α, and AFP. Graph represents one of five similar experiments expressed as mean ± SD.

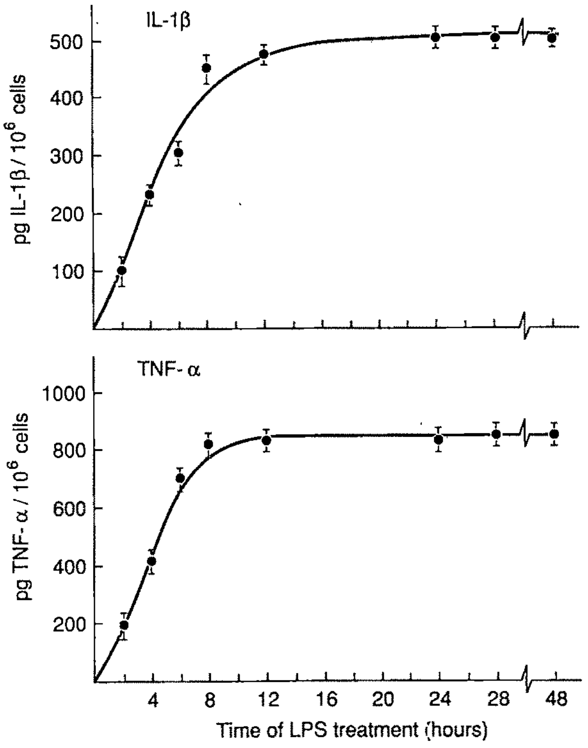

Fig. 4. Time course of IL-1β *(top)* and TNF-α *(bottom)* production by lipopolysaccharide-treated Kupffer cells. After overnight culture, adherent Kupffer cells (1 × 10^6) were washed, then treated with 10 μg/ml of lipopolysaccharide, and quantities of IL-1β and TNF-α were measured by enzyme-linked immunosorbent assay at indicated time points. Figure represents one of five similar experiments expressed as mean ± SD.

tivate Kupffer cells. After incubation for 24 hours with 0.1 to 20 μg of lipopolysaccharide per milliliter, the quantities of TNF-α and IL-1β released into culture supernatants were measured by enzyme-linked immunosorbent assay (Fig. 5). Amounts of TNF-α were noted to increase with as little as 0.1 μg/ml of lipopolysaccharide, with a large increase observed at 0.5 μg/ml of lipopolysaccharide, and a plateau response between 5 and 20 μg of lipopolysaccharide per milliliter (Fig. 5, bottom). Similarly, IL-1β increased in a dose-dependent fashion with a plateau response between 5 and 20 μg of lipopolysaccharide per milliliter (Fig. 5, top).

Effect of growth factors and cytokines in Kupffer cell production of TNF-α (Fig. 6). To determine whether other factors have the potential to activate Kupffer cells, we incubated purified cells with specific growth factors and interleukins for 24 hours and measured the quantities of TNF-α released into culture supernatants. As shown in Fig. 6, IL-1α and IL-1β were able to stimulate Kupffer cells at concentrations of approximately 1.0 ng/ml (10^{-9} to 10^{-10} mol/L). Because several recent reports indicate that IL-6 may serve as an intermediate for IL-1 action in certain cell types,[25] we tested the ability of IL-6 to activate fetal Kupffer cells. The Kupffer cells could also be stimulated to release TNF-α when incubated with recombinant IL-6, TNF-β, and interferon gamma, but these required 20- to 500-fold higher concentrations. The stimulatory effect on the release of TNF-α was demonstrated to occur in a dose-dependent fashion for each interleukin or interferon shown. Endotoxin levels of each recombinant factor were determined to be <0.2 ng/10 μg of cytokine by the manufacturer. Using a modification of the *Limulus* amebocyte lysate assay capable to detecting picogram quantities of endotoxin contamination,[24] we were unable to detect lipopolysaccharide in any of the cytokines tested. We were unable to detect any TNF-α production after incubation with epidermal growth factor (200 ng/ml), platelet derived growth factor (200 ng/ml), insulin-like growth factor I (200 ng/ml), insulin-like growth factor II (200 ng/ml), or IL-3 (200 ng/ml).

Comment

Studies of the functional capacities of human fetal Kupffer cells are limited. Because of our interest in the development of the human fetus and particularly in the function of fetal liver, we felt that it was important to develop isolation techniques for the study and characterization of fetal Kupffer cells. We report here, for the first time, the methods for isolation of human fetal Kupffer cells to >90% purity. We have characterized these cells by morphologic features, by α-naphthyl acetate esterase staining, and by immunofluorescence staining with mouse monoclonal antibodies known to react with human macrophages and monocytes.[26] We do not believe that the preparations were contaminated with peripheral monocytes since <0.5% of the adherent cells were recognized by anti-Leu-M1, a mouse monoclonal antibody characterized to react with 90% of circulating human monocytes but not with tissue macrophages.[27] In addition, <1% of our adherent cells were recognized by three monoclonal antibodies that detect high-density determinants on human T cells.

On the basis of our estimates approximately 1% to 2% of the cells present in human fetal liver at 13 to 19 weeks' gestation are Kupffer cells. Our estimates are based on α-naphthyl acetate esterase staining and immunofluorescence staining with mouse monoclonal antibody 3C10 on frozen sections and touch preparations of intact fetal liver. These estimates agree with results obtained from a morphometric analysis of rat liver sections by electron microscopy, which determined that 2.1% of liver volume was occupied by Kupffer cells.[5] In addition, studies demonstrate that total hematopoietic cells can account for a significant proportion of the fetal liver mass during the tenth to twentieth gestational week; approximately 1% to 2% of the cells evaluated were macrophages.[7] Similarly, when frozen tissue sections from human fetal liver at 10 to 20 weeks were stained with a panel of mouse monoclonal antibodies reactive with class II antigens, (which recognize cells of either macrophage or lymphoid histologic type but not parenchymal cells or red blood cell precursors), estimates of positive cells ranged between 0.3% and 7.7%.[28] Some of the variability in the estimates of Kupffer cell number has been attributed to macrophage heterogeneity in the fetal liver as a function of maturation.[29] These findings indicate an evolving maturational process of Kupffer cells at 10 to 20 weeks' gestation and could explain our observation that 5% of the adherent cells failed to be recognized by any of the monoclonal antibodies against macrophages or monocytes used in this study.

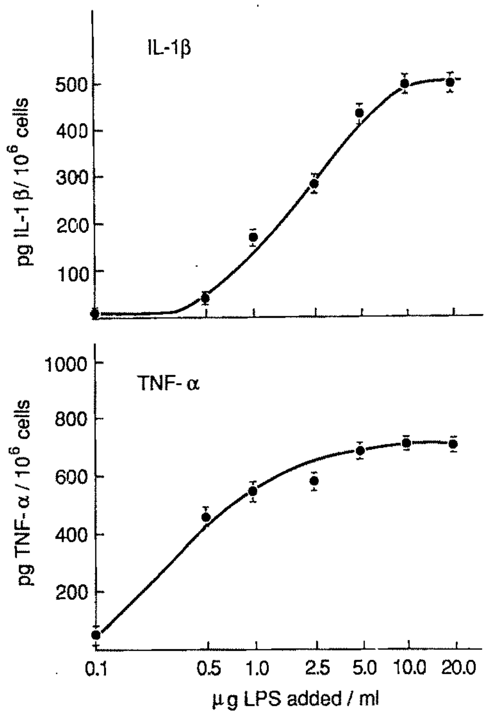

Fig. 5. Dose-response of IL-1β *(top)* and TNF-α *(bottom)* production by lipopolysaccharide-treated Kupffer cells. After overnight culture, adherent Kupffer cells (1 × 10^6) were washed, then treated with indicated amounts of lipopolysaccharide for 24 hours, and quantities of IL-1β and TNF-α released into culture supernatants were measured by enzyme-linked immunosorbent assay. Figure represents one of five similar experiments expressed as mean ± SD.

Functionally, Kupffer cells at 13 to 19 weeks' gestation were able to actively ingest 0.8 μm latex beads in vitro after a 30-minute incubation at 37° C. We have been unable to isolate intact fetal liver from specimens collected before 13 weeks' gestation in quantities sufficient to determine their functional ability. Phagocytosis of particles >0.1 μm has been described as an important criterion to distinguish Kupffer cells from other sinusoidal cells of the liver.[30] In agreement with our findings of active phagocytosis, cells from midgestation human fetal liver that stained positive with OKM-1, a mouse monoclonal antibody to human macrophages, apparently had phagocytosed maternal immunoglobulins or immune complexes, since they contained both immunoglobulin light chain markers, κ

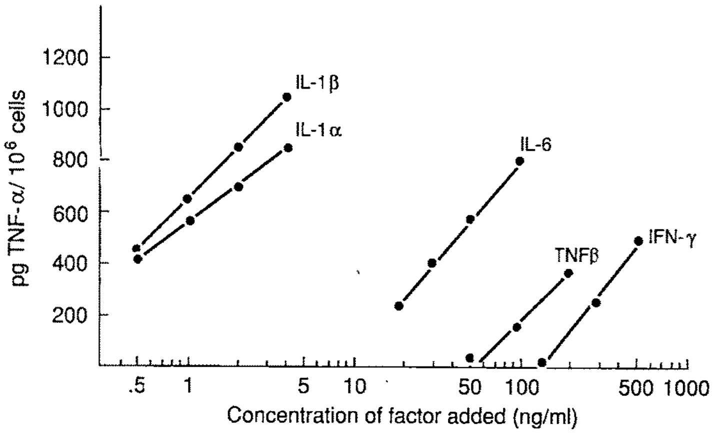

Fig. 6. Effects of several cytokines on Kupffer cell production of TNF-α. Adherent Kupffer cells (1 × 10^6) were incubated for 24 hours with indicated amounts of recombinant IL-1β, IL-1α, IL-6, TNF-β, or IFN-γ, and amounts of TNF-α released into culture supernatants were determined by enzyme-linked immunosorbent assay. Figure represents one of five similar experiments expressed as means. For clarity of publication standard deviations are not shown.

and λ, a situation not described for individual lymphocytes.[31]

Purified fetal Kupffer cells used in this study were stimulated to release both TNF-α and IL-1β with lipopolysaccharide in a dose-dependent fashion (Fig. 5). Similar results were obtained by other investigators when fetal and newborn rat hepatic macrophages were treated with lipopolysaccharide.[16] Similarly, TNF-α and IL-1β were secreted when cord blood monocytes from human preterm and term infants were treated for 24 hours with 10 μg lipopolysaccharide per milliliter.[18] The time course for lipopolysaccharide-stimulated production of TNF-α and IL-1β by human fetal Kupffer cells was similar to those reported by others for adult rat[14, 32] and murine Kupffer cells,[14] human[33] mononuclear phagocytes, and fetal and newborn rat hepatic macrophages.[16] We suggest that the functional activities of human fetal Kupffer cells at 13 to 19 weeks' gestation, including phagocytosis and the production of TNF-α and IL-1β, may be important in the developing fetus. IL-1 has been shown to have direct effects on hepatocytes, endothelial cells, fat-storing (Ito) cells, and Kupffer cells.[34] TNF-α, which has multiple paracrine actions on hepatocytes,[35] may act through specific binding sites that are present on mouse liver membrane preparations.[36]

We sought to identify other factors, in addition to lipopolysaccharide, that might play important roles in vivo by stimulating the production of TNF-α from human fetal Kupffer cells. Both IL-1α and IL-1β were able to stimulate the release of TNF-α when added to fetal Kupffer cells at a concentration of 10^{-9} to 10^{-10} mol/L. IL-1, which mediates the body's response to microbial invasion and inflammation, represents a well-characterized activator of macrophage function.[37] Tamatani et al.[38] have identified IL-1α and IL-1β in normal amniotic fluid of 16- to 18-week pregnancies, indicating the potential of these cytokines to alter fetal Kupffer cell function.[38] The multifunctional cytokines, TNF-α, IL-1α, IL-1β, and IL-6 are produced by macrophages and also have been shown to activate macrophage function.[39] Thus human fetal Kupffer cells would have the potential of being exposed to systemically derived or locally produced cytokines. Although these cells display mature macrophage function in vitro, these data do not provide direct evidence for any in vivo phenomena.

Recent evidence from adult human and animal systems suggests that cytokines may alter hepatocyte function and cholesterol metabolism in vivo by paracrine interactions.[40-42] This is particularly important in the fetus where the liver is the major source of cholesterol in the fetal circulation.[43, 44] Multiple cytokines may alter liver growth and cholesterol metabolism. It has recently been demonstrated that TNF-α, IL-1β and interferon alfa all stimulate hepatic fatty acid synthesis in a rapid fashion, whereas the same cytokines increase hepatic cholesterol synthesis in a delayed fashion.[42] In addition, IL-1β is a potent inhibitor of hepatocyte cell division.[45] These observations, coupled with our findings that TNF-α and IL-1β are produced in human fetal Kupffer cells, suggest a possible role of these factors in human hepatocyte function and cholesterol metabolism by paracrine and autocrine interactions.

In summary, we have purified and cultured human fetal Kupffer cells and demonstrated that these cells possess mature macrophage function. We suggest possible roles of these cells in the clearance of bacterial products, autocrine regulation of immune mechanisms, and paracrine regulation of other immune cells, hepatocytes, and adipocytes. Further work is in progress to determine how these Kupffer cells are activated and how their products might communicate with and regulate cell function in the developing fetus.

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Endothelin production by amnion and its regulation by cytokines

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Cells derived from human amnion, an avascular tissue, produced significant amounts of endothelin 1 and 2, although the rate of biosynthesis was approximately one tenth of that of human umbilical vein endothelial cells. Endothelin 1 and 2 was not produced in measurable amounts by cells derived from chorion laeve or decidua. Production of endothelin 1 and 2 by amnion cells was increased by treatment with the cytokines interleukin-1β, tumor necrosis factor, and interleukin-6. The biosynthesis of endothelin 1 and 2 by an avascular tissue is suggestive of a wider role for endothelins than has been recognized. (AM J OBSTET GYNECOL 1991;165:120-4.)

Key words: Endothelin, amnion, cytokine, avascular

Endothelins are a family of small proteins that were originally isolated from the culture supernatants of porcine aortic endothelial cells.[1] Endothelin 1 was originally reported to be a potent vasoconstrictor, and expression of the endothelin 1 gene was found to be regulated by several vasoactive agents. Endothelins 1, 2, and 3 have since been described; endothelin 1 is porcine-human endothelin and endothelin 3 is rat endothelin.[2-4] These 21-residue peptides are formed from a preproendothelin (~200 residues) by way of a 38/39-residue intermediate.[1, 2] It is thought that endothelins act as endogenous agonists of dihydropyridine-sensitive calcium channels.[1] Included among an increasing array of properties of endothelins are the induction of two types of contractions of the rat uterus,[5] activation of phospholipase A,[6] and activation of phospholipase C.[7, 8] Additionally, endothelins recently have been found to be synthesized not only by endothelial cells but also by kidney cells.[9] We have recently found that endothelin 1 is present in amniotic fluid[10] and that its concentration is elevated in amniotic liquor from women with infections of the amniotic fluid who are delivered preterm. This study was undertaken to evaluate potential sources of endothelin 1 within the uterus and to determine whether inflammatory mediators regulate the rate of production of this protein.

Material and methods

Placentas were obtained at cesarean section at term before the onset of labor. Amnion was separated manually and decidua was scraped from the maternal face

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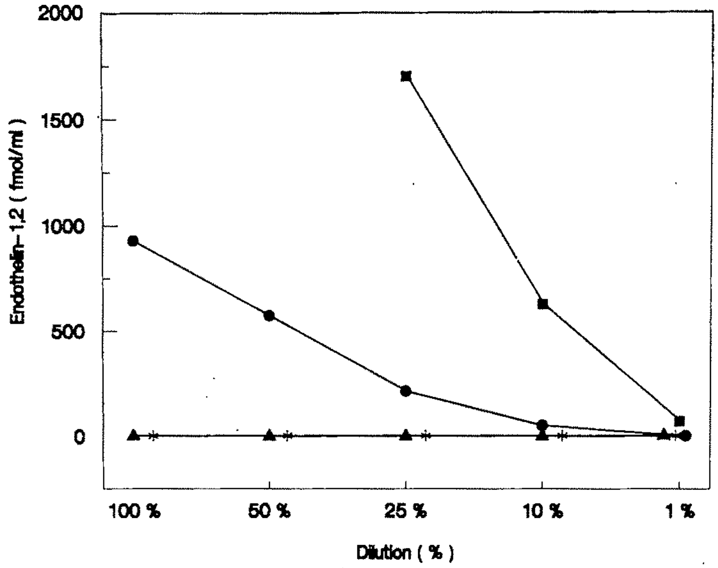

Fig. 1. Endothelin 1 and 2 in dilutions of culture media from amnion, chorion, and decidual cells (initial measurements are concentrates from 50 ml of conditioned media) and umbilical vein endothelial cells (initial measurements are concentrates from 10 ml of conditioned media). *Circles,* Amnion; *triangles,* chorion; *asterisks,* decidual cells; *squares,* umbilical vein endothelial cells.

of the chorion. Cells were prepared from amnion as described previously.[11] Chorion laeve cells were dispersed by treatment with collagenase and isolated by separation of the dispersate on a discontinuous Percoll gradient.[12] Decidual cells also were prepared by collagenase dispersion and isolated on a different discontinuous Percoll gradient.[13] Endothelial cells were prepared from umbilical cords according to the method of Jaffe.[14] Cells were grown to confluence, at which time media for analyses were obtained.

In most experiments culture media were used without manipulation. In one series of experiments culture media were subjected to anisotropic ultrafiltration through a filter with a molecular weight cutoff of 10,000, followed by concentration of the filtrate on a C_{18} Sep Pak (Millipore Corp., Milford, Mass.).[15] Interleukin-1β was purchased from Amgen Biologicals (Thousand Oaks, Calif.); tumor necrosis factor and interleukin-6 were obtained from R & D Systems (Minneapolis).

Endothelin 1 and endothelin 1 and 2 were measured by means of radioimmunoassay kits from Peninsula Laboratories, Inc. (Belmont, Calif.) and Amersham Corporation (Arlington Heights, Ill.), respectively. Cellular protein was determined by the method of Lowry et al.[16] Statistical differences were assessed by Fisher's least significant difference test.

Results

Measurements of endothelin 1 and 2 in serial dilutions of concentrated conditioned media clearly demonstrated secretion of immunoassayable material, not only by endothelial cells (as expected) but also by amnion cells (Fig. 1). Conditioned media from chorion and decidual cells did not contain detectable amounts of endothelin 1 and 2. To establish the relative secretion rates of endothelin 1 and 2 by endothelial and amnion cells, we determined its basal rate of production by confluent monolayers from several individual tissues (Fig. 2). Endothelin 1 and 2 production by endothelial cells was approximately tenfold greater than that of amnion cells.

The antiserum used in the studies at this point, although raised against endothelin 1, cross reacts 200% with endothelin 2 (Amersham Corporation). Hence we could have been measuring either or (more likely) both proteins. We deemed it important to be more specific in our measurements and to evaluate production of endothelin 1 alone. First, we compared measurements of endothelin 1 and endothelin 1 and 2 in conditioned media of amnion cells from several individual tissues under a variety of conditions (Fig. 3). The results indicated a significant correlation between measurements by each method, with endothelin 1 and 2 levels being greater. Although many interpretations of these data are possible, the most obvious is that amnion cells secrete both endothelin 1 and endothelin 2, with endothelin 1 secretion being greatest quantitatively. To evaluate regulation of endothelin 1 production alone, we used the endothelin 1–specific radioimmunoassay (Peninsula Laboratories) in the next series of studies.

Incubation of amnion cells with interleukin-1β and

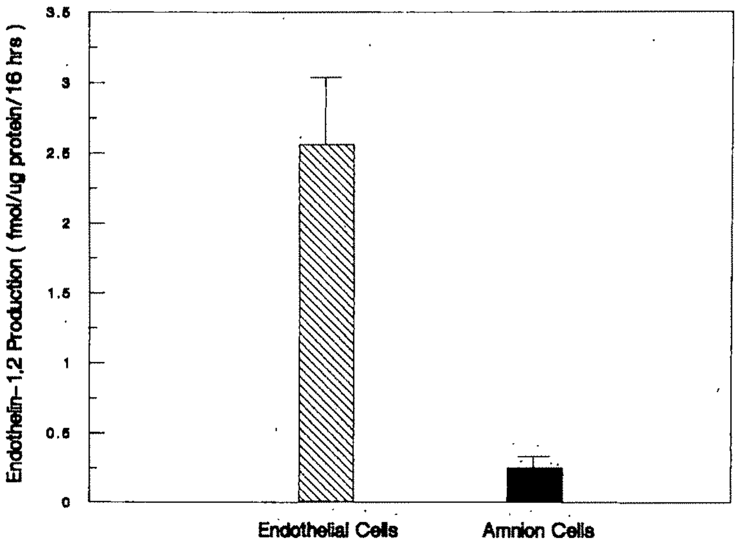

Fig. 2. Endothelin 1 and 2 production (mean ± SEM) by endothelial cells ($n = 6$) and amnion cells ($n = 9$). Difference is statistically significant, $p < 0.005$.

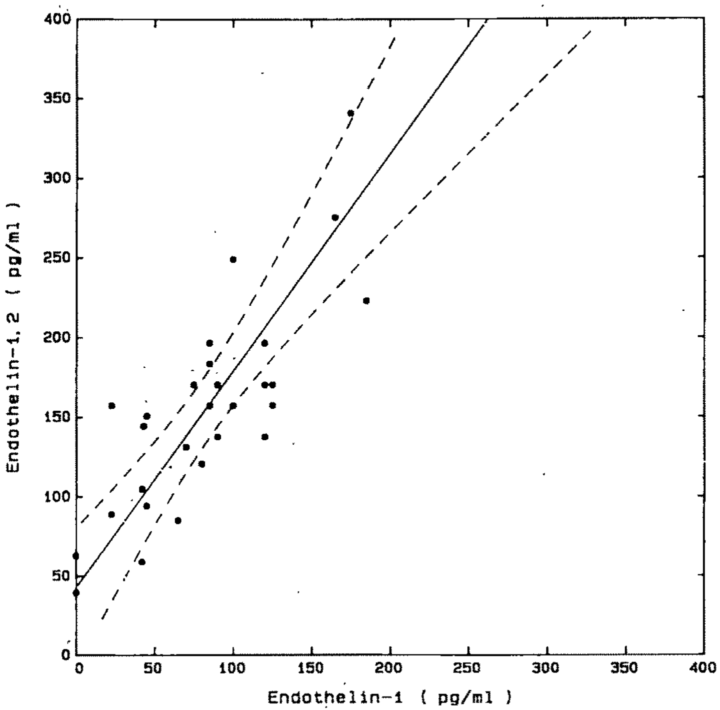

Fig. 3. Comparison of measurements of endothelin 1 and 2 versus endothelin 1 in amnion cell culture media. Linear regression analysis with y = endothelin 1 and 2 and x = endothelin 1 gave $y = 1.69x + 15$; $r = 0.80$.

tumor necrosis factor induced significant increases in the rate of production of endothelin 1 (Fig. 4, *A* and *B*). The results from representative experiments are presented. Similar results have been obtained from experiments with cells from five individual tissues. In contrast, incubation of amnion cells with interleukin-6 resulted in variable effects on endothelin 1 production, although the highest concentration of interleukin-6 used always stimulated endothelin 1 secretion (Fig. 4, *C*). The reasons for this variability are uncertain.

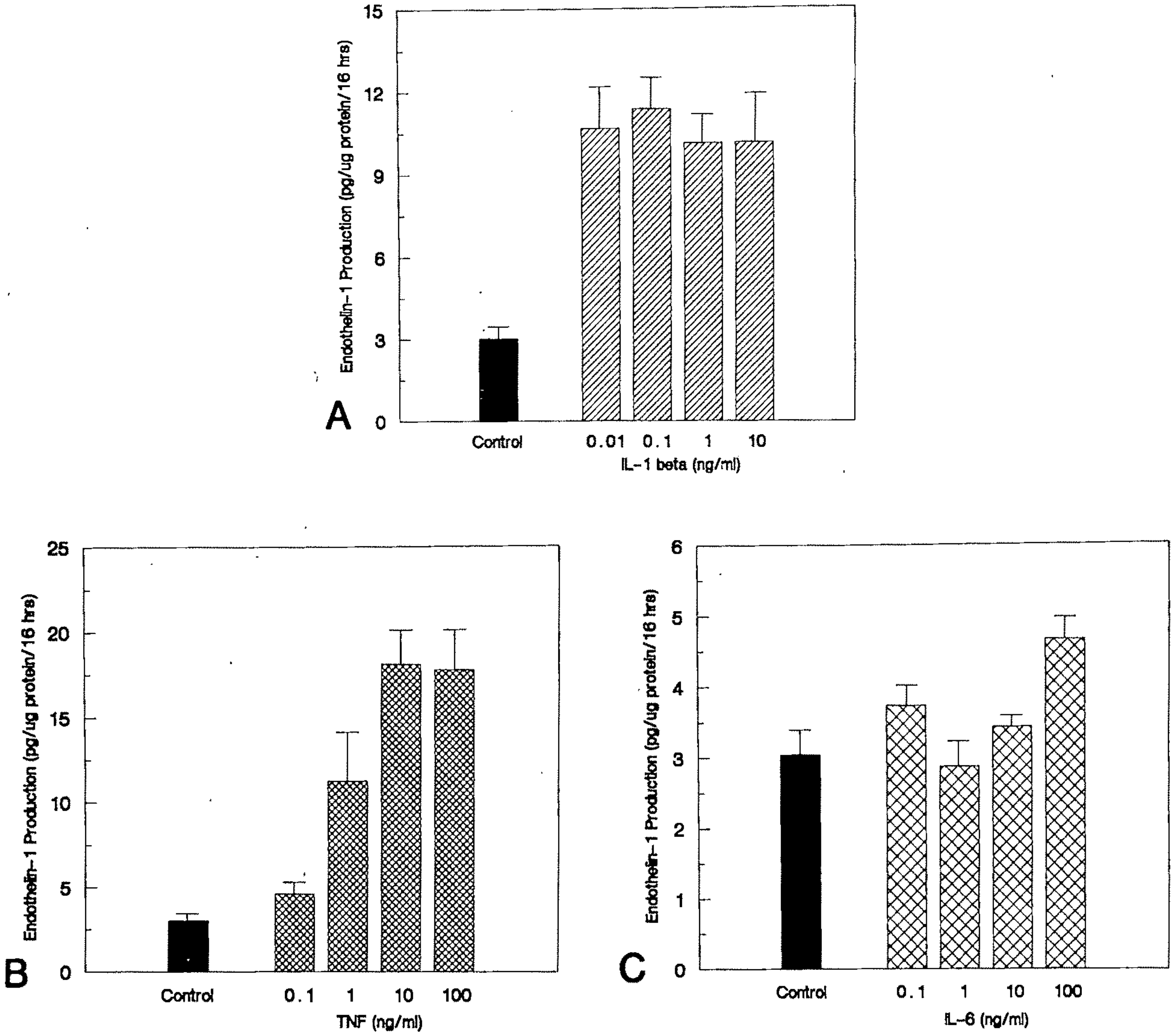

Fig. 4. Effects of cytokines on endothelin 1 production by amnion cells. **A,** Interleukin-1β *(IL-1 beta)*; **B,** Tumor necrosis factor *(TNF)*; **C,** Interleukin-6 *(IL-6)*. Control versus interleukin-1β, $p < 0.005$ (all concentrations). Control versus tumor necrosis factor, $p < 0.005$ (all concentrations). Control versus interleukin-6, $p < 0.002$ at 100 ng/ml.

Similar findings were obtained with endothelin 1 and 2 measurements after cytokine stimulation of amnion cells.

Comment

Our results indicate that endothelin 1 and probably endothelin 2 are synthesized and secreted by avascular amnion. However, it will be important to determine that these proteins have bioactivity, as well as immunoreactivity, and that they are produced by fresh amnion tissue, as well as by cells derived from the tissue. Nevertheless, the data suggest that endothelin production may be more widespread than previously thought. Endothelin production by some cells and tissues may be missed as a result of difficulties in the assay procedure. In our original studies[17] we were often misled by significant matrix effects of different culture media in the assay. Careful attention to the control of these effects was necessary to permit the correct determination of endothelin production by amnion cells.

The increase in amnion endothelin 1 production induced by cytokines is consistent with our finding of raised amniotic fluid levels of endothelin 1 and 2 in pregnancies complicated by intrauterine infections.[10] Moreover, these findings are also consistent with the increase in endothelin messenger ribonucleic acid expression in amnion cells induced by interleukin-1.[18] What role(s) could amnion-derived endothelin 1 serve during pregnancy? Endothelin 1 has been shown to be potent in both elevating cytoplasmic calcium concentrations and increasing myosin phosphorylation in human myometrium.[19] Hence, if endothelin secreted by amnion could reach the myometrium, it could have a significant effect on contractile function. More speculatively, it is possible that endothelin participates in the mechanisms governing amniotic fluid volume. Overall, therefore, our finding of endothelin 1 production by amnion may be of great significance to our future understanding of certain physiologic changes with pregnancy and parturition in women.

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Uterine and fetal Doppler flow changes after intravaginal prostaglandin E_2 therapy for cervical ripening

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Nineteen patients received prostaglandin E_2 as a 2.5 mg gel (seven cases), 3.3 mg chip (five cases), or 10 mg controlled-release pessary (seven cases) for preinduction cervical ripening. Blood flow velocities did not change significantly for the uterine arcuate artery, fetal aorta, and umbilical artery circulations after any of the treatments. In no case did systolic/diastolic ratios consistently elevate above baseline values. (AM J OBSTET GYNECOL 1991;165:125-6.)

Key words: Prostaglandin E_2, Doppler flow, uterine artery, umbilical artery

Low doses of prostaglandin E_2 (PGE_2) instilled intravaginally have become more widely used for preinduction cervical ripening in complicated pregnancies with an unfavorable cervix. Novy et al.[1] reported in the ewe model that PGE_2 exerts a vasoconstrictive effect on the placental bed, decreases umbilical blood flow, and increases fetal arterial pressure. Information is needed in human beings on the effects of PGE_2 on uterine and fetal hemodynamics.

After the study was approved by our Institutional Review Board, gravidas with singleton fetuses at term (37 to 43 weeks' gestation) were enrolled if induction of labor was required and an unfavorable cervix (Bishop score ≤ 4) was present. We have described the measurement of pulsed Doppler flow by the same technician (L.A.) with an aTL Ultramark 4 machine with a mechanical 3.5 mHz transducer.[2] Uterine artery, fetal aortic, and umbilical artery waveforms were sought through use of a combined pulsed real-time B-mode system that allowed precise location for Doppler recording on a real-time image.

After baseline circulatory recordings were obtained, 19 participants were assigned to receive one of three vaginal doses of PGE_2: 2.5 mg in a gel (seven cases), 3.3 mg in a chip (five cases), or 10 mg in a controlled-release pessary (seven cases) as part of a randomized drug trial. Serum concentrations of the principal metabolite of PGE_2 were expected to peak within the first hour, so all measurements were made 15 minutes before dosing, immediately thereafter, and at 15-minute intervals for 2 hours. The flow (velocity-time) waveforms were examined visually and quantitated. A systolic/diastolic (S/D) ratio was then calculated by measuring the ratio of peak systolic Doppler shift to least diastolic Doppler flow. Statistical comparisons were made with an analysis of variance.

Doppler fetal and uterine hemodynamic parameters were not significantly affected by the PGE_2 treatments. The baseline arcuate artery S/D ratio was 1.78 ± 0.28 (mean ± 1 SD), and after PGE_2 instillation the ratio was 1.82 ± 0.34 ($p = 0.45$). The umbilical artery S/D ratio was 2.56 ± 0.60 before PGE_2 administration and 2.46 ± 0.46 after therapy ($p = 0.32$). The fetal aortic velocity was 45.9 ± 14.0 cm/sec and 41.8 ± 11.5 cm/sec before and after PGE_2 treatment, respectively ($p = 0.81$). The power of this study to detect a 25% or greater change in the fetal or uterine Doppler blood flow was 90%. A comparison of PGE_2 chip, pessary, and gel therapies by multiple Student's t tests did not reveal significant differences among any of the modes of drug administration.

In no case was the baseline uterine arcuate artery S/D ratio greater than 2.5. Three fetuses had averaged baseline umbilical artery S/D ratios of 3.0 or greater (3.21, 3.25, 3.81). In no case did absolute values increase or remain elevated on two or more consecutive recordings.

Findings from this investigation revealed no change in the uterine and fetal circulations after short-term exposure to a single intravaginal dose of PGE_2. This observation was true when a presumably rapidly absorbed gel or chip was used. We would assume that the lack of local blood flow change with the controlled-release pessary would continue beyond the first 2 hours after dosing. Results in this study in human beings with the use of a pulsed Doppler system differ from obser-

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vations by Novy et al.[1] in the fetal lamb with radiolabeled microspheres. In that study the fetal bradycardia and hypotension found within seconds were followed by hypertension and vasoconstriction of the umbilical circulation shortly thereafter. Furthermore, relatively higher doses of PGE_2 were used, which resulted in higher concentrations than are found in human maternal and fetal plasma at birth.

All 19 of our study cases had pregnancy complications that predisposed the patients to uteroplacental insufficiency. We were reassured to observe no vasopressor effects after PGE_2 dosing, even in the three cases with a previously documented elevated umbilical artery flow resistance. Our observations were limited to term pregnancies, so conclusions cannot be made about the blood flow changes in premature fetuses. This investigation also does not address the safety of sequential doses, but we would assume that this would be safe with adequate time between dosings. As with any drug that may stimulate uterine contractions, close monitoring of uterine activity and the fetal heart rate remain essential.

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Does intensive glycemic control in diabetic pregnancies result in normalization of other metabolic fuels?

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Intensive treatment of insulin-dependent diabetes mellitus during pregnancy often normalizes plasma glucose levels. However, it is unclear whether this adversely affects other metabolic fuels that are essential to normal fetal growth and development. Metabolic studies were conducted after the subjects ingested a standardized mixed meal during each trimester in 7 normal and 15 insulin-dependent diabetic pregnant women. The latter were treated with continuous subcutaneous insulin infusion or multiple injections, which were adjusted to achieve strict glucose control throughout pregnancy. Insulin, alanine, branched-chain amino acids, triglycerides, free fatty acids, and ketones were measured every 15 to 30 minutes before a standardized breakfast and for 150 minutes after the breakfast. Patients with insulin-dependent diabetes mellitus were studied while they received their usual insulin dosages. Fasting glucose levels (87 ± 7 mg/dl) and glucose levels 150 minutes after the meal (112 ± 11 mg/dl) were near normal. However, normoglycemia was achieved at the expense of increased plasma insulin levels (area under insulin response curves, $p < 0.01$, vs nondiabetic curves). Nevertheless, fasting and post-prandial plasma branched-chain amino acids, alanine, and free fatty acids were similar in both groups. Fasting cholesterol, triglyceride, and ketone levels were also normalized. We conclude that normalization of circulating amino acids and lipids in conjunction with correction of hyperglycemia may contribute to favorable outcomes in infants of intensively treated diabetic mothers. (AM J OBSTET GYNECOL 1991;165:126-30.)

Key words: Diabetes, pregnancy, treatment, amino acids, lipids

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Marked abnormalities in fuel metabolism are frequently seen in patients with insulin-dependent diabetes who receive conventional insulin therapy. Those abnormalities are probably a result of inadequate insulin replacement. This type of insulin deficiency commonly leads to fasting and postprandial hyperaminoacidemia and hyperlipidemia, as well as hyperglycemia; these effects are compounded in pregnancy by insulin antagonist hormones elaborated by the placenta. Excessive delivery of substrates to the fetus and responsive fetal hyperinsulinemia are generally accepted as important factors that contribute to adverse pregnancy outcomes in poorly controlled diabetic mothers.[1, 2]

Aggressive insulin therapy with either multiple daily injections or continuous subcutaneous insulin infusion is recommended for treating pregnant women with diabetes. Most diabetic women who are strongly motivated to test blood glucose levels frequently (which permits appropriate insulin adjustment) can achieve substantially lower blood glucose values during pregnancy. This treatment prevents or ameliorates some problems faced by the infant of the mother with poorly controlled diabetes. Maintenance of strict treatment goals requires subcutaneous administration of steadily increasing doses of insulin that lead to peripheral hyperinsulinemia and that could, in turn, have adverse metabolic effects. However, relatively little attention has been paid to the effects of intensive insulin treatment during pregnancy on substrates other than glucose. To examine this issue, we measured circulating concentrations of amino acids and lipids both before and after a standardized mixed meal during each trimester of pregnancy in women with and without insulin-dependent diabetes. Our findings suggest that normal concentrations of insulin-sensitive metabolic fuels remain available to the developing fetus of the diabetic mother throughout pregnancy, despite maternal hyperinsulinemia.

Material and methods

Subjects. Fifteen women with C-peptide–negative insulin-dependent diabetes mellitus were studied. These women were recruited from patients referred to the Yale Diabetes-in-Pregnancy Program for intensive insulin treatment. They used either multiple daily injections or continuous subcutaneous insulin infusion. The patients ranged in age from 24 to 32 years (mean ± SD, 28 ± 2 years), in duration of disease from 2 to 21 years (16 ± 4 years), and in White's class from B to FR. They were in good health, were taking no medications besides insulin, vitamins, and iron supplements, and were between 6 and 24 weeks' gestation when they entered the study. Twelve of the 15 patients were studied during each trimester, and the remaining 3 were studied only during the second and third trimesters. Seven patients were treated with multiple daily injections, and eight patients received continuous subcutaneous insulin infusion therapy. Details of outpatient diabetes management and obstetric care have been reported elsewhere.[3] Seven nondiabetic control subjects with an age range similar to that of the pregnant diabetic group were enlisted from a group of healthy pregnant nurses working at the Yale–New Haven Hospital. They were taking no medications other than vitamins and iron, had no family history of insulin-dependent diabetes mellitus or gestational diabetes, and had normal glucose tolerance tests during routine screening at 24 to 28 weeks. Three meal studies (one during each trimester) were performed on each of the control subjects.

Meal study protocol. All meal studies were begun between 7 and 8 AM after an 8- to 10-hour overnight fast. An indwelling catheter was inserted in an antecubital vein, and blood was obtained for measurement of glycosylated hemoglobin, basal substrate, and insulin after a 30-minute rest period. Each subject then ingested a standard mixed breakfast meal prepared by the dietary staff of the Yale General Clinical Research Center. The meal contained 12 kcal/kg of body weight and consisted of 50% carbohydrate, 30% fat, and 20% protein. The meal was designed to approximate what the patients with insulin-dependent diabetes mellitus had been advised to eat at home. Fifteen minutes before they ate, all patients received the same prebreakfast bolus insulin dose that they usually took at home; patients with continuous subcutaneous insulin infusion were maintained on their preprogrammed basal insulin doses. Additional blood samples were obtained for substrate and insulin measurements 30, 90, and 150 minutes after meal ingestion. First-trimester meal studies were performed between 10 and 12 weeks' gestation; second-trimester studies, between 26 and 28 weeks; and third-trimester studies, between 34 and 36 weeks.

Determinations of metabolites. Plasma glucose was measured with a glucose analyzer (Beckman Instruments, Fullerton, Calif.). Standard laboratory techniques were used to determine serum cholesterol and triglycerides, and plasma free fatty acids were assayed by the procedure of Novak.[4] Plasma amino acids were determined by column chromatography,[5] and glycosylated hemoglobin was measured chromatographically with a microcolumn method (Isolab, Akron, Ohio). The reference range in healthy nonpregnant, nondiabetic women for the glycosylated hemoglobin assay was 5% to 8%. Plasma insulin was measured by a double-antibody radioimmunoassay.[6] In the diabetic patients free insulin was measured by precipitating plasma samples at the bedside with polyethylene glycol.[7] Blood ketones were measured by enzymatic assay, and

Table I. Fasting lipid and ketone levels in diabetic and nondiabetic subjects during pregnancy

Subjects	*Cholesterol (mg/dl)*	*Triglycerides (mg/dl)*	*Ketones (μmol/L)*	*Glycosylated hemoglobin*[*] *(%)*
First trimester				
Control	180 ± 19	119 ± 14	0.15 ± 0.10	—
Insulin-dependent diabetes mellitus	171 ± 9	87 ± 11	0.20 ± 0.03	7.6 ± 0.2
Second trimester				
Control	218 ± 28[†]	165 ± 18[†]	0.10 ± 0.03	—
Insulin-dependent diabetes mellitus	220 ± 10[†]	157 ± 13[†]	0.17 ± 0.10	6.5 ± 0.2[†]
Third trimester				
Control	228 ± 23[†]	174 ± 19[†]	0.07 ± 0.05	—
Insulin-dependent diabetes mellitus	237 ± 8[†]	215 ± 16[†]	0.17 ± 0.23	6.4 ± 0.1[†]

[*] Normal range, 5.5% to 8.0%.

[†] $p < 0.05$ versus first-trimester value.

all measurements were performed within 1 month of study.[8]

Statistical analysis. The data were analyzed to determine the quantitative (area under the curve) and qualitative (shape or pattern of the curve) response of each metabolite to a mixed meal. Repeated-measures analysis of variance was performed; the area under the curve was corrected for baseline differences as the dependent variable. Because the method of insulin replacement (multiple daily injections vs continuous subcutaneous insulin infusion) did not affect the results, data in all subjects with insulin-dependent diabetes mellitus were combined for comparison with data in normal controls. The analysis assessed group differences (subjects with insulin-dependent diabetes mellitus vs controls) and trimester differences. If a significant difference was observed throughout pregnancy by the analysis, the Student t test was used to determine in which trimester (or trimesters) a difference occurred. To examine the pattern of response within each trimester, analysis of variance was also performed; the level of the metabolite at each time period was used as the dependent variable. The analysis was done with the general linear model procedure in the SAS (Statistical Analysis Systems, Cary, N.C.) statistical package. Data are presented as means ± SE.

Results

Mean glycosylated hemoglobin levels were within the range of normal in the patients with insulin-dependent diabetes mellitus during each trimester (Table I) in association with mean fasting and postprandial glucose levels, which also were within our target values (plasma glucose levels, ≤100 mg/dl fasting and ≤120 mg/dl at 2 to 3 hours after a meal) (Fig. 1). Plasma insulin profiles in nondiabetic and diabetic subjects are shown in Fig. 2. In nondiabetic subjects plasma insulin concentrations rose promptly to peak values between 60 and 75 U/ml 30 minutes after the meal and returned to baseline levels by 150 minutes. In contrast, insulin levels rose more gradually in patients with insulin-dependent diabetes mellitus after the premeal insulin bolus (at −15 minutes) and remained above the basal value after 150 minutes. The area under the curve and the pattern of response between patients with insulin-dependent diabetes mellitus and controls were significantly different throughout pregnancy ($p < 0.01$).

Despite differences in plasma insulin, basal and postprandial levels of alanine and branched-chain amino acids (isoleucine, leucine, and valine) were similar in women with insulin-dependent diabetes mellitus and in nondiabetic women during each trimester (Fig. 2). In addition, circulating concentrations of other essential amino acids, including phenylalanine, methionine, and threonine, were not different from normal in the patients with diabetes throughout pregnancy (data not shown). In both diabetic and nondiabetic subjects the area under the curve for alanine increased significantly during pregnancy ($p < 0.01$), and in both groups the integrated area for branched-chain amino acids fell during pregnancy ($p < 0.05$). Basal free fatty acids were slightly (but not significantly) higher in the diabetic pregnant subjects than in the nondiabetic pregnant subjects throughout pregnancy (Fig. 2). Nevertheless, the fall in fatty acids after the meal was similar in both groups. The area under the free fatty acid curve increased significantly in both groups during pregnancy and was greater in the diabetic group than in the control group during the three trimesters ($p < 0.02$).

As summarized in Table I, fasting concentrations of cholesterol and triglycerides increased significantly in both subjects with insulin-dependent diabetes mellitus and in nondiabetic subjects during pregnancy, but there were no significant differences between the groups for either of these lipid fractions. Fasting ketone levels did not differ between the groups or from baseline values within each group during pregnancy (Table I).

Comment

It has become clear in recent years that embryogenesis and fetal development are significantly influenced by the metabolic environment in which the embryo and

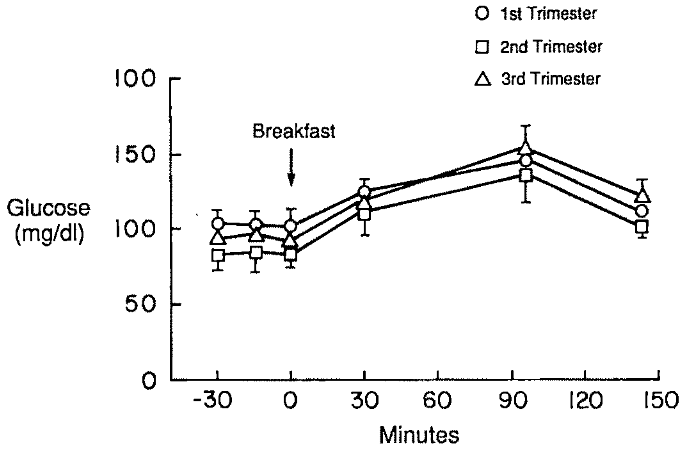

Fig. 1. Glucose profile before and after breakfast in first, second, and third trimesters.

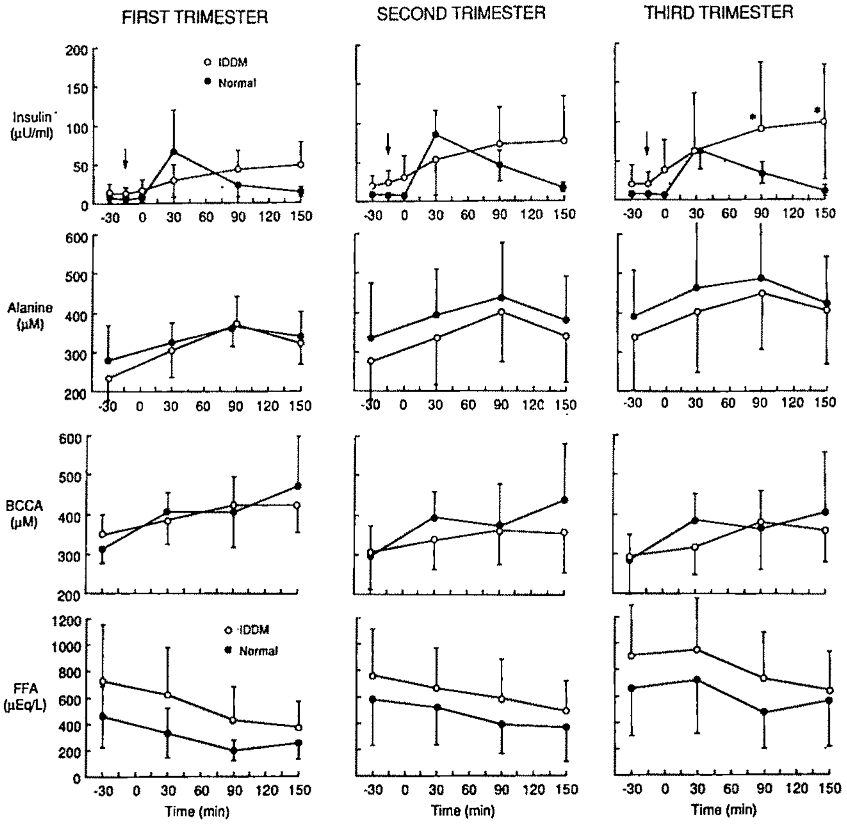

Fig. 2. Metabolic profiles before and after mixed breakfast of insulin, alanine, branched-chain amino acids, and free fatty acids in normal patients and in patients with insulin-dependent diabetes mellitus.

fetus develop. Investigations primarily confined to the effects of hyperglycemia on the developing fetus have shown that adverse outcome results from embryonal or fetal exposure to hyperglycemia. Such exposure during organogenesis leads to malformations[9-11] and is associated with increased perinatal mortality during the third trimester.[12] Experimental studies also have shown that ketone bodies have deleterious effects on the developing embryo.[13] Diabetes mellitus, with derangement of various metabolic fuels (carbohydrates, amino acids, lipids, and ketones), has the potential to adversely affect the developing fetus. Therefore strict glycemic control in pregnancy has become the standard of care in modern obstetrics.

As this study indicates, very strict glycemic control can be achieved in women with insulin-dependent diabetes mellitus during pregnancy via either multiple daily insulin injections or continuous subcutaneous insulin infusion by mechanical pump.[3] Near-normal glycemic control was achieved in our patients, even in the presence of plasma insulin profiles that differed substantially from those observed in nondiabetic pregnant women. Both forms of insulin administration deliver the hormone into the peripheral circulation via the subcutaneous route; therefore it is not surprising that patients with diabetes showed a delayed and markedly prolonged rise in plasma insulin levels when compared with those of nondiabetic subjects. As a result, the integrated insulin area was significantly increased in the diabetic subjects versus the nondiabetic subjects.

A goal of the study was to determine whether failure to precisely simulate plasma insulin profiles in pregnant diabetic women with intensive insulin therapy had adverse consequences for insulin-sensitive metabolic fuels other than glucose. On one hand, peripheral hyperinsulinemia might lead to greater suppression of circulating substrates and might limit their availability to the fetus. Alternatively, the delayed absorption of insulin from the subcutaneous site might fail to correct exaggerated postprandial increments in such substrates, which could contribute to persistent problems, such as macrosomia, in the fetus.[14-16]

It is especially noteworthy that normal basal and postprandial branched-chain amino acid levels were maintained throughout pregnancy in our patients with insulin-dependent diabetes mellitus. Intensive insulin therapy prevented the hyperaminoacidemia (particularly that involving the branched-chain amino acids) that is characteristic of poorly controlled insulin-dependent diabetes mellitus. Furthermore, aggressive insulin therapy in our pregnant patients with insulin-dependent diabetes mellitus was not associated with a fall in plasma branched-chain amino acid levels to subnormal, as was reported in some studies of intensive treatment of nonpregnant patients with insulin-dependent diabetes mellitus.[2, 17] These amino acids are important because they are primarily responsible for muscle nitrogen repletion and are capable of stimulating protein synthesis in vitro. Circulating concentrations of the other essential amino acids, alanine (a key gluconeogenic substrate), and ketones also were maintained within the range seen in pregnant nondiabetic women via intensive insulin therapy during pregnancy. Although free fatty acid levels remained slightly increased in the patients with insulin-dependent diabetes mellitus, these treatments did not significantly alter the changes in fasting cholesterol and triglycerides that were observed in the nondiabetic women during pregnancy.

In conclusion, the use of intensive insulin therapy in pregnant patients with insulin-dependent diabetes mellitus is associated with near normalization (and not suppression) of insulin-sensitive metabolites that are available to the fetus despite the presence of maternal hyperinsulinemia. Infants born to women in this study were free of complications, such as macrosomia, that commonly result from poorly controlled diabetes during pregnancy.[3] It is noteworthy that Kalkhoff et al.[18] have recently shown a direct relationship between maternal plasma amino acid concentrations and birth weight in infants of diabetic and nondiabetic mothers. Thus the influence of intensive insulin therapy on amino acid, lipid, and glucose metabolism may play a role in the generally favorable outcomes of pregnancy that result from these treatments.

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The enhanced production of placental interleukin-1 during labor and intrauterine infection

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The purpose of this study was to determine the effect of labor and chorioamnionitis in interleukin-1 production by human placenta. We studied the activity of the placenta to produce interleukin-1 with an enzyme immunoassay by culturing tissue blocks. The placental tissue obtained after labor produced a larger amount of interleukin-1 than placental tissue obtained before labor. All the placental tissues produced more interleukin-1β than interleukin-1α. The placentas with labor and chorioamnionitis produced about seventeenfold more interleukin-1 than placentas with labor only. We immunohistochemically identified interleukin-1–producing cells in the placenta and found that syncytiotrophoblasts produced both interleukin-1α and interleukin-1β, while Hofbauer cells produced only interleukin-1β. In vitro analysis of the trophoblast activities to produce interleukin-1 revealed that microbial byproducts enhanced interleukin-1 production, possibly inducing accumulation of interleukin-1 receptor–positive cells at the sites of inflammation. In addition to stimulation of prostaglandin biosynthesis and labor, the placental interleukin-1 may act as an inflammatory mediator, leading to systemic and local changes at fetomaternal interface and activating fetomaternal immune systems against intrauterine infection. (AM J OBSTET GYNECOL 1991;165:131-7.)

Key words: Placental interleukin-1, trophoblast, labor, intrauterine infection

A growing body of evidence indicates the contribution of many factors in the onset of human labor.[1-4] Such factors include bacterial endotoxin,[1] bacterial phospholipases,[2] and some cytokines, including interleukin-1[3] (IL-1) and tumor necrosis factor-α,[4] in the case of intrauterine infection. However, our knowledge of the mechanism of the onset of labor in the absence of infection remains incomplete.

IL-1 is a 17 kd glycoprotein that is produced by various kinds of cells, including macrophages.[5] It plays important roles in a number of biologic responses, such as stimulation of the immune response, induction and regulation of inflammatory disease, stabilization of physiologic homeostasis, promotion of cellular proliferation, and differentiation of various cellular systems.[5] Biochemical and molecular analysis of IL-1 has identified two kinds of molecules with similar functions: IL-1α and IL-1β.[6] They perform their unique functions through the same receptors.[7]

IL-1 is one of the most potent regulators of prostaglandin biosynthesis by intrauterine tissue.[3, 4, 8] It also acts as a strong modulator of immune and inflammatory responses.[5] The source of interuterine IL-1 that is responsible for the onset of labor and the mechanisms for and significance of enhanced production of IL-1 in the presence of intrauterine infections are poorly understood. The objectives of the studies described in the present communication therefore were: (1) to measure, by enzyme immunoassay, placenta-derived IL-1α and IL-1β produced by placental explants with or without labor, (2) to examine whether explants with intrauterine infection such as chorioamnionitis show enhanced IL-1 production, (3) to immunohistochemically identify the cells producing placental IL-1, and (4) to analyze the immunoreactivity of trophoblasts in vitro. Our study demonstrates the important significance of placental IL-1 during labor and intrauterine infection in parturition.

Material and methods

Reagents. Human recombinant IL-1α and recombinant IL-β were kind gifts from Dainippon Pharmaceutical Co. (Osaka) and Dr. Y. Hirai of Otsuka Pharmaceutical Co. (Tokushima, Japan). Lipopolysaccharide was purchased from Sigma Chemical Co. (St. Louis). Concanavalin A and *Staphylococcus aureus* Cowan I were purchased from Pharmacia Fine Chemical Co. (Uppsala).

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Preparation of tissue explants. For preparation of placental tissue, normal term placentas without medical complications were obtained after vaginal delivery or at elective cesarean section after 38 to 41 weeks' gestation. Immediately after delivery or operation, the decidual tissue was carefully removed and the placenta was minced into 1 mm^3 pieces with sterile forceps. The placentas with chorioamnionitis were prepared similarly. The clinical criteria of chorioamnionitis were as follows: a high fever before delivery with or without other known sites of infection, maternal and fetal tachycardia, uterine tenderness, leukocytosis, and a foul odor of the amniotic fluid.[9] To confirm chorioamnionitis, histopathologic studies of the placentas were performed, as described previously.[9] Histopathologic examination of the placenta and cord demonstrated polymorphonuclear leukocyte or neutrophil infiltration, initially in the extraplacental membranes and later in the chorionic plate and adjacent intervillous space. Control blocks, including ovaries and uteri, were obtained by simple hysterectomy with adenectomy and prepared in a similar way. After thorough washing, the blocks were incubated in a 24-well flat bottom microplate (Corning, Iwaki Glass, Tokyo) at 37° C in a humidified incubator with 5% carbon dioxide in RPMI 1640 medium (Gibco, Grand Island, N.Y.) containing 10% fetal calf serum (Hyclone, Logan, Utah), 100 U/ml penicillin, and 100 μg/ml streptomycin. The culture medium and fetal calf serum were endotoxin-free as determined by the *Limulus* amebocyte lysate test.[3] After incubation the culture was centrifuged at 200 *g* for 10 minutes. The supernatants were removed, filtered through a 0.22 μm Millipore filter (Millipore, Bedford, Mass.), and frozen immediately at −80° C until the time of assay for IL-1. At the end of the incubation period, the tissue was removed and dried completely to determine the dry weight of the tissue. The titers of IL-1 produced by the blocks were expressed as picograms per 1 ml per 1 mg of dry weight of the tissue.

Titration of IL-1 by enzyme immunoassay. The IL-1 titers in the supernatant were determined by enzyme immunoassay kits with monoclonal antibodies that were specific for each of human IL-1α (ANOC 301) and IL-1β (ANOC 205) (Otsuka Assay Research Foundation, Tokushima).[10] The kits are capable of detecting >10 pg/ml of IL-1α and 20 pg/ml of IL-1β, respectively. Interassay and intrassay variabilities of the IL-1α were 4.2% to 6.9% and 6.7% to 7.5%, while those of the IL-1β were 4.2% to 9.5% and 6.7% to 8.9%. Cross-reactivity between IL-1α and IL-1β is not observed. Neither enzyme immunoassay kit is able to detect any of the following cytokines: IL-6, IL-2, tumor necrosis factor-α, granulocyte macrophage colony-stimulating factor, macrophage colony-stimulating factor, interferon beta, or interferon gamma. They are able to detect the exact amount of IL-1 molecules in the presence or absence of fetal calf serum but not in human serum.

Immunohistochemical analysis of IL-1 in the placenta. To determine the localization of IL-1 molecules in the placenta, an immunohistochemical technique was applied, by means of the avidin-biotin-peroxidase complex method (PK-4002, Vectastatin Laboratories, Burlingame, Calif.), as described previously.[11] Briefly, after washing with phosphate-buffered saline solution, either the normal placental block or the block of placenta with chorioamnionitis was quickly frozen in liquid nitrogen. Cryostat sections of the blocks were air-dried, followed by fixation in cold acetone (100%) for 20 minutes and rinsing with phosphate-buffered saline solution. The sections were first bleached in 0.3% hydrogen peroxide to block endogenous peroxidase and then covered with 2% normal horse serum to minimize nonspecific binding. The appropriately diluted monoclonal anti-IL-1α (ANOC 301) and anti-IL-1β antibodies (ANOC 205) were applied at 4° C overnight. After rinsing with phosphate-buffered saline solution, the sections were further incubated for 30 minutes with biotin-labeled horse antimouse immunoglobulin G followed by the addition of avidin-peroxidase complex at 4° C. The sites of peroxidase activity in the sections were visualized with 0.1% 3,3-diaminobenzidine-tetrahydrochloride containing 0.02% hydrogen peroxidase in 0.1 mol/L Tris buffer (pH 7.2). The slides were counterstained lightly with Mayer's hematoxylin.

Preparation of single-cell suspensions of placental cells. To functionally determine the cell suspensions that produce IL-1, single cell suspensions of placental cells were prepared as described previously.[12] Briefly, a placental block was cultured in deoxyribonuclease type I (Sigma) and 0.125% trypsin (Sigma) for 30 minutes at 37° C, followed by application onto a Percoll sedimentation gradient and centrifugation for 15 minutes at 1800 revolutions per minute. Three separate cell populations were obtained. After thorough washing, the middle fraction (5×10^5/ml), which predominantly consisted of trophoblast cells, was resuspended and cultured in 24-well flat-bottom microplates (2 ml per well) (Corning) with fetal calf serum. All the leukocytes and red blood cells in the placenta were collected in the bottom fraction. To further reduce the number of contaminating macrophages, the cells in the middle fraction were cultured at 37° C for 1 hour to let macrophages adhere the plastic dishes.[13] The middle fraction was tested for contamination by macrophages,

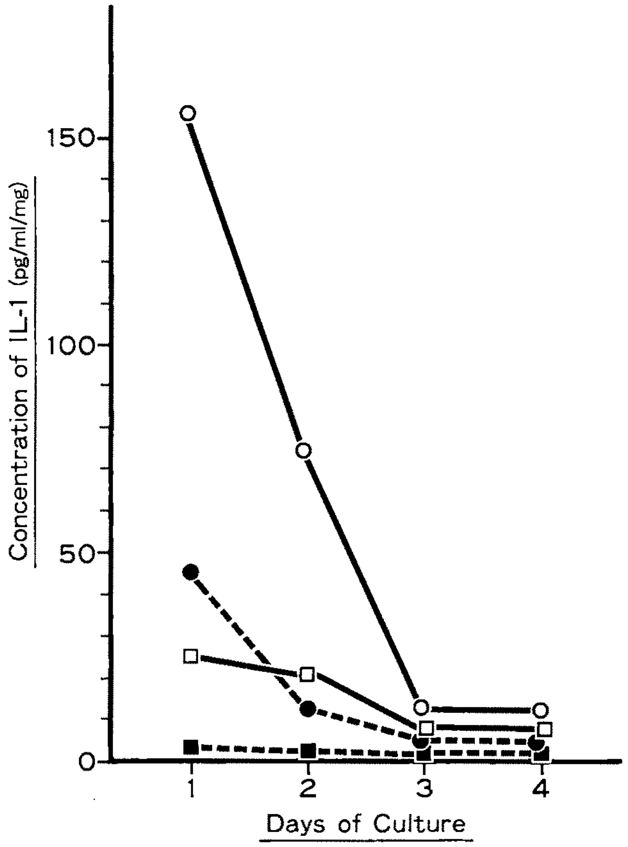

Fig. 1. Time course of IL-1α and IL-1β production by placental blocks with labor ($n = 3$) or without labor ($n = 3$). Blocks were prepared from placental tissues obtained before or after labor. Media were changed every 24 hours and cell-free culture supernatants of the blocks were collected each day. Titers of IL-1α and IL-1β in supernatants were determined by enzyme immunoassay as described in Material and methods. *Open symbols* indicate IL-1α (□——□) and IL-1β (○——○) from placentas with labor, and *solid symbols* represent IL-1α (■——■) and IL-1β (●——●) from placentas without labor. Each titer represents mean; SE was within 15%.

T cells, and B cells by immunofluorescence analysis with KP-1 anti-CD 68 (Dakopatts, Glostrup), Leu 4 anti-CD 3 (Becton-Dickinson, Mountain View, Calif.), and B1 anti-CD 20 monoclonal antibodies (Coulter Clone, Hialeah, Calif.), respectively.

Stimulation of trophoblasts with mitogens. The cells in the trophoblast-enriched fraction were adjusted to 5×10^5/ml and stimulated with the various concentrations of mitogens originally used for the activation of macrophages (lipopolysaccharide), T cells (concanavalin A), and B cells (*S. aureus* Cowan I).[13] Cell-free culture supernatants were collected and filtered at 24, 48, and 72 hours after the stimulation. The culture supernatants were then frozen at −80° C until used for titration of IL-1.

Statistical analysis. The statistical significance of experimental differences was assessed with Student's *t* test for paired data.

Results

Fig. 1 shows the time course of titers of IL-1α and IL-1β produced by the placental blocks obtained before and after labor (each group, $n = 3$). Further culture of either blocks resulted in a decline in both IL-1 titers, showing the highest activity of placentas to produce IL-1 at 24 hours.

Fig. 2 shows that the placental blocks with labor ($n = 15$) produced a statistically larger amount of IL-1α ($p < 0.05$) and IL-1β ($p < 0.05$) than those without labor ($n = 16$) for 24-hour culture. Either block produced predominantly IL-1β rather than IL-1α.

The explants with labor and chorioamnionitis ($n = 3$) produced 328.7 ± 85.3 pg/ml/mg of IL-1α and 1489.0 ± 831.0 pg/ml/mg of IL-1β, titers, which were about seventeenfold higher than those of the blocks with labor only.

Fig. 3 shows the immunohistochemical profile of cell

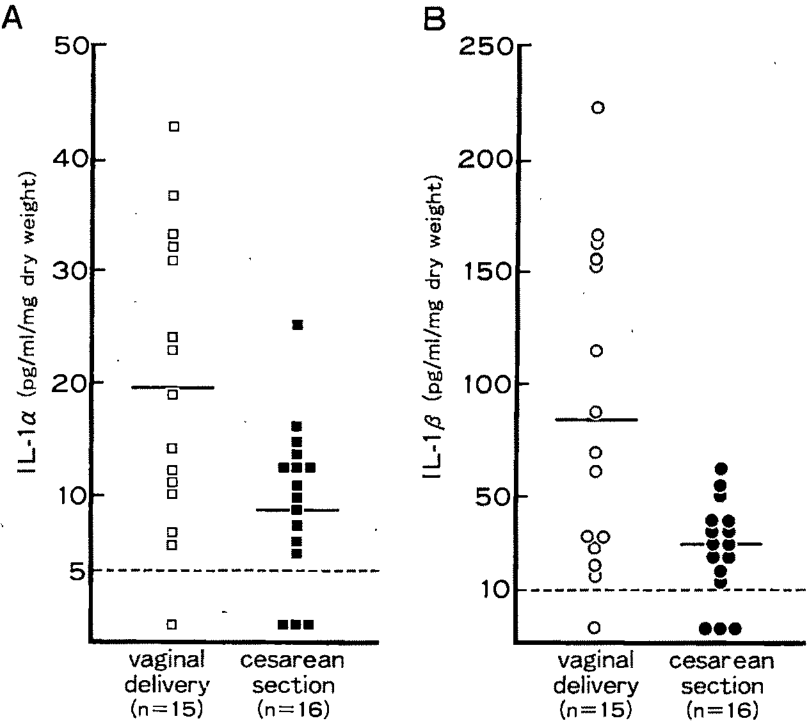

Fig. 2. Production of IL-1α (**A**) and IL-1β (**B**) by placental blocks with or without labor. Supernatants, cultured for 24 hours, were assayed for IL-1α and IL-1β by enzyme immunoassay. *Open symbols* indicate IL-1α (□) and IL-1β (○) from placental blocks with labor. *Solid symbols* represent IL-1α (■) and IL-1β (●) from blocks without labor. *Horizontal dashed bar* represents mean of each experimental group. *Horizontal dotted bar* indicates threshold (5 pg/ml/mg for IL-1α and 10 pg/ml/mg for IL-1β) of assay.

populations in the placenta with labor that produced IL-1α and IL-1β. The outer trophoblast layer, i.e., the syncytiotrophoblast, was positively stained with a monoclonal anti-IL-1α antibody (Fig. 3, *A*). Other cell populations, such as mesenchymal cells and endothelial cells in the placenta, were negative for staining. In contrast, an anti-IL-1β antibody showed that syncytiotrophoblasts and some Hofbauer cells prodcued IL-1β (Fig. 3, *B*). The similar findings were obtained with the explants without labor or with chorioamnionitis (data not shown).

Enhanced production of IL-1 in chorioamnionitis led us to investigate the activity of trophoblasts to produce IL-1 in vitro. Purified normal trophoblasts spontaneously produced a small amount of IL-1 in unstimulated conditions. The trophoblasts showed maximal production of IL-1 for 2 days of culture in response to lipopolysaccharide at doses from 10^{-2} to 10 μg/ml (Fig. 4, *A*) and to *S. aureus* Cowan I at doses from 10^{-4} to 10^{-1} (vol/vol) (Fig. 4, *B*). In contrast, the trophoblasts failed to produce any increased amount of IL-1 by the stimulation with concanavalin A (Fig. 4, *C*). The titer of IL-1α produced by trophoblasts was generally higher than that of IL-1β under these conditions. Purified trophoblasts contained <3% contamination by $CD68^+$ macrophages, $CD3^+$ T cells, and $CD20^+$ B cells, as determined by immunofluorescence analysis. Preterm trophoblasts showed similar immunocompetent activity to produce IL-1 by mitogenic stimulation (data not shown).

Comment

Decidual IL-1 is one source of intrauterine IL-1 and is produced by endotoxin-activated macrophage-like cells.[14] We demonstrated that the placental IL-1 is another source of the intrauterine IL-1. Indeed, there have been some reports demonstrating the presence of placental IL-1 detected by a bioassay for IL-1.[15,16] However, no reports have examined the cause-effect relationship between placental IL-1 and the presence of labor with or without intrauterine infection. We demonstrated the relationship by titration of placental IL-1 with enzyme immunoassay, because IL-1 is composed of two distinct species and the bioassay for IL-1 is easily interfered with by an IL-1 inhibitor that is often present in samples.[17] Furthermore, we immunohistochemically determined the source of IL-1α from syncytiotrophoblasts and IL-1β from syncytiotrophoblasts and Hofbauer cells.

Placentas with labor produced a larger amount of

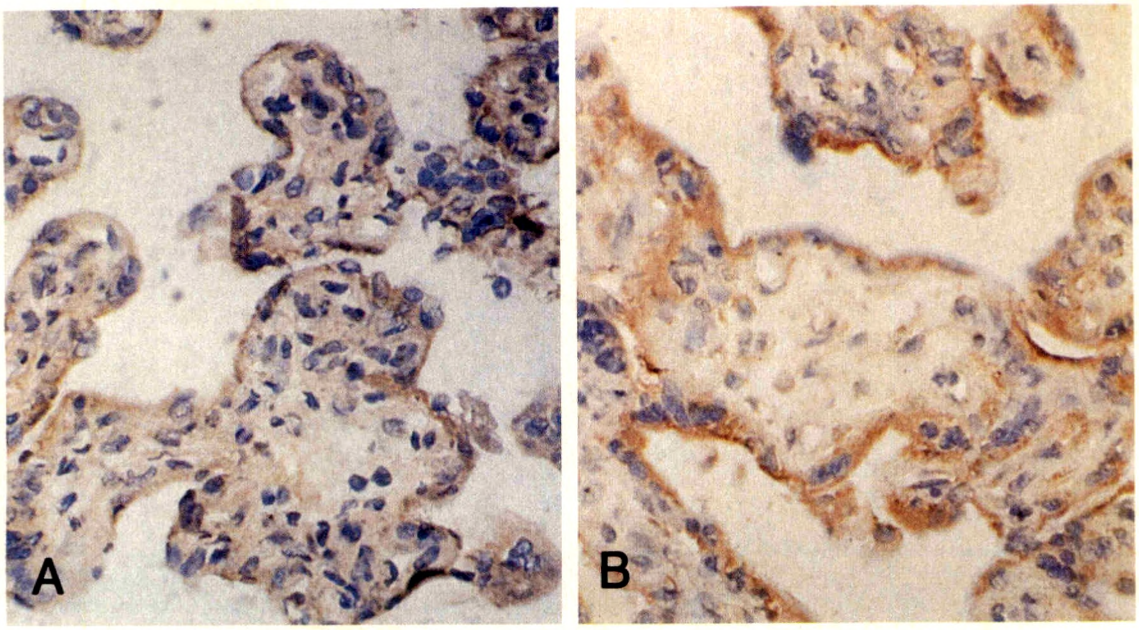

Fig. 3. Immunohistochemical demonstration of IL-1–producing cells in placenta. Freshly frozen placental explants with labor were prepared and stained with mouse monoclonal antibodies against **(A)** human IL-1α (ANOC 301) and **(B)** human IL-1β (ANOC 205) by the avidin-biotin method; they were subsequently counterstained with Meyer's hematoxylin. (Original magnification, ×400.)

IL-1 than the placentas without labor, showing maximal IL-1 production at 24 hours. It is less likely that the placentas with labor produced a higher amount of IL-1, because of the stimulation by bacteria in the vagina and the fact that the placentas were cultured in an endotoxin-free condition. The result was also not due to cell death since there was no significant difference of cell viability between the groups, as determined by the trypan blue dye exclusion test of single cell suspensions from each group. Rather, it is more likely to suppose that the placenta actively produces a baseline level of IL-1 in vivo and that the elevated placental IL-1 directly stimulates the biosynthesis of prostaglandins in the decidua, leading to the onset of spontaneous term labor and delivery. It is unclear, however, what signals regulate placental IL-1 production in an infection-free condition.

The role of IL-1 in amniotic fluid has been suggested to be the signal for the onset of parturition.[3] The intraamniotic IL-1 might be derived from desquamated fetal keratinocytes or decidual cells in infection.[3] However, we showed another possibility that intraamniotic IL-1 is also derived by syncytiotrophoblasts and Hofbauer cells because of transmembrane passage of IL-1[3] from the placenta into the amniotic fluid. Therefore our data support the hypothesis that the placental IL-1 might contribute to the enhanced concentration of intraamniotic IL-1 during labor or intrauterine infection, because the placenta showed increased production of IL-1 in that condition. Thus placental IL-1 might be also involved in stimulation of prostaglandin biosynthesis by amniotic cells, leading to the onset of labor and delivery in another way.

The regulation of IL-1α and IL-1β production has been shown to be controlled by distinct molecular mechanism in IL-1–producing cells.[18] Placental blocks, with or without labor or intrauterine infection, produced a larger amount of IL-1β than IL-1α. However, purified trophoblasts secreted more IL-1α than IL-1β under mitogen-free and mitogen-stimulated conditions. The different pattern with placental blocks might be due to deletion of Hofbauer cells during the in vitro procedure for purification of the trophoblasts. There are also some discrepancies between placental IL-1 level and that of IL-1 in amniotic fluid. IL-1α is higher than IL-1β in amniotic fluid of women with labor, whereas IL-1β is dominant in that of women with preterm labor in intrauterine infection.[3] Complex mechanisms might operate to regulate the fluctuation of IL-1 levels in amniotic fluid.

The systemic and local inflammatory changes in chorioamnionitis might be induced by the actively secreted placental and decidual IL-1[14] with synergistic action of a number of other chemotactic factors.[19] IL-1 acts as a chemotactic agent to induce the accumulation of IL-1 receptor–positive neutrophils at the site of inflammation.[20] The activated neutrophils secrete proteases such as elastase, collagenase, phospholipid A_2, and oxygen radicals, which act as cytocidal agents against invading bacteria.[19] The enhanced production of intrauterine

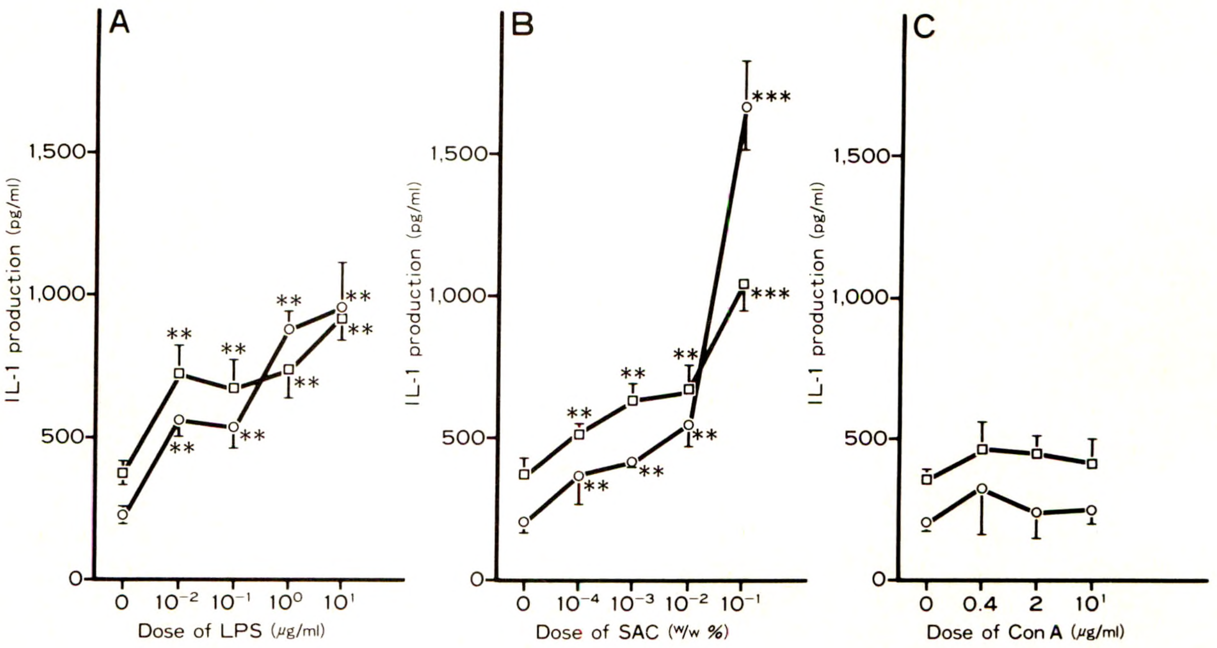

Fig. 4. Production of IL-1α and IL-1β by purified trophoblasts stimulated with mitogens. Trophoblasts were enriched by Percoll density gradient after enzyme (deoxyribonuclease type I and trypsin) treatment of placental blocks without labor. Purified trophoblasts (5×10^5/ml) were stimulated with various concentrations of lipopolysaccharide **(A)**, *S. aureus* Cowan I **(B)**, and concanavalin A **(C)**. After 48-hour culture, cell-free culture supernatants were collected and assayed for IL-1α (□) and IL-1β (○) by enzyme immunoassay. Data represent mean ± SE of triplicate cultures. Three different experiments showed identical results. *Double asterisk*, $p < 0.01$ versus control; *triple asterisk*, $p < 0.001$ versus control.

IL-1 in chorioamnionitis also may activate the fetomaternal immune systems, because T cells and B cells that also express IL-1 receptor are activated, proliferated, and differentiate under such conditions,[21] thus potentiating host (fetal and maternal) immune responses against infections and reducing fetal morbidity and mortality.

By stimulation of purified trophoblasts with mitogens, we developed an in vitro model to investigate the immunologic activities of trophoblasts in the setting of intrauterine infection. Trophoblasts augmented their IL-1 production in response to microbial byproducts including lipopolysaccharide and *S. aureus* Cowan I, both of which would be released by gram-negative[19] and gram-positive microorganisms in intrauterine infection, respectively. Although preparation of purified trophoblasts resulted in contamination by macrophages, T cells, and B cells, the amount of IL-1 potentially produced by these contaminating monocytes, rather than by trophoblasts, was negligible. Trophoblasts showed high responsiveness to both mitogens, whereas human macrophage responds only to lipopolysaccharide and B cells only to *S. aureus* Cowan I.[13] Since trophoblasts can be activated by bacterial endotoxin (lipopolysaccharide) to produce IL-1 in a similar way to the decidual cells,[11] our in vitro experiments with trophoblasts might explain, at least in part, the mechanism responsible for the increased IL-1 production by placentas with chorioamnionitis. Thus the experimental system would be useful for the biochemical and molecular analysis of trophoblast-mediated immunologic functions in intrauterine infection.

IL-1 plays a critical role in initiation and augmentation of host immune systems[5, 22] and possesses the unique activities to stimulate biosynthesis of prostaglandins[3, 4, 8]; therefore we propose that placental IL-1 contributes to potentiation of fetomaternal immune responses against intrauterine infection and promotion of labor not only in term but also in preterm parturition.

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Fibrinolytic parameters in normotensive pregnancy with intrauterine fetal growth retardation and in severe preeclampsia

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In pregnancy a decrease in fibrinolytic activity, which is due to an increase in plasminogen activator inhibitor activity and plasminogen activator inhibitor type 1 and type 2, has been described. Because the placenta is a source of both type 1 and type 2 plasminogen activator inhibitor, we have studied them and other fibrinolytic parameters in a group of normotensive pregnant women with intrauterine fetal growth retardation and in two groups of women with preeclampsia, with or without intrauterine growth retardation. A significant increase in plasminogen activator inhibitor type 1 antigen and plasminogen activator inhibitor activity was observed in preeclampsia, with or without intrauterine growth retardation, but not in normotensive pregnancy with intrauterine growth retardation, when compared with normal pregnancy. Plasminogen activator inhibitor type 2 antigen levels showed a significant decrease in both groups of pregnant women (normotensive or preeclamptic) with intrauterine growth retardation when compared with pregnancies without intrauterine growth retardation. A significant correlation between plasminogen activator inhibitor type 2 levels and fetal weight has been observed in the clinical groups. (AM J OBSTET GYNECOL 1991;165:138-42.)

Key words: Intrauterine growth retardation, preeclampsia, fibrinolysis, plasminogen activator inhibitor

It is generally accepted that perinatal mortality is increased in pregnancies with intrauterine fetal growth retardation (IUGR).[1]

Occlusive lesions in placental vasculature and decidual spiral arteries, named *acute atherosis* or *atheromatous-like lesions*, can be found in normotensive pregnancies complicated by IUGR and pregnancy-induced hypertension (preeclampsia).[2, 3]

A decrease in fibrinolytic activity that is due to an increase in plasminogen activator inhibitor activity, plasminogen activator inhibitor type 1 and type 2, has been described in normal pregnancy.[4-10] Moreover, a depressed fibrinolytic status caused by high levels of plasminogen activator inhibitor type 1 has been described in patients with preeclampsia,[9, 10] and in a previous report[9] we described elevated levels of type 1 in plasma that are positively correlated with the severity of placental damage. On the other hand, reduced levels of plasminogen activator inhibitor type 2 in plasma in patients with preeclampsia have been reported.[9, 11]

Although preeclampsia is commonly associated with small-for-gestational-age infants, IUGR may occur without hypertensive disease, and the severity of fetal growth retardation is correlated with the degree of placental infarction.[3]

Because the placenta is a key source of type 1 and type 2 plasminogen activator inhibitor, we have studied them and other fibrinolytic parameters in normal pregnancy, in normotensive pregnancy with IUGR, and in patients with preeclampsia with and without IUGR.

Material and methods

Clinical groups. The normotensive pregnancy with IUGR group comprised 11 normotensive pregnant women who were diagnosed as having suspected IUGR when serial ultrasonographic measurements of biparietal diameter and femur length were >2 SD below the mean for gestational age two consecutive times in a month.[12] After delivery, diagnosis of IUGR was confirmed when newborn weight was below the 10th percentile of appropriate birth weight for gestational age and neonatal ponderal index was <2.20 (2.01 ± 0.10; range, 1.90 to 2.19).

Sixteen primigravid patients with no previous history of hypertension in whom severe preeclampsia developed during the third trimester comprised the two preeclamptic pregnancy groups. Severe preeclampsia was

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Table I. Clinical data

	Intrauterine growth retardation (n = 11)	*Matched controls* (n = 26)*	*Preeclampsia with IUGR (n = 10)*	*Matched controls* (n = 17)*	*Preeclampsia without IUGR (n = 6)*	*Matched controls* (n = 26)*
Age (yr)						
Mean ± SD	29.0 ± 7.7	28.6 ± 5.4	26.7 ± 4.4	27.2 ± 6.5	29.3 ± 6.3	28.6 ± 5.4
Range	19-42	20-42	21-36	20-42	21-37	20-42
Gestational age at sampling (wk)						
Mean ± SD	37.2 ± 1.7	37.5 ± 2.5	33.7 ± 2.1	33.6 ± 1.8	38.3 ± 2.2	37.5 ± 2.5
Range	34-40	34-42	30-37	30-36	34-40	34-42
Gestational age at delivery (wk)						
Mean ± SD	37.7 ± 1.7	39.5 ± 1.8	34.0 ± 2.4	39.2 ± 1.6	38.7 ± 1.4	39.5 ± 1.8
Range	34-41	36-42	30-38	36-40	36-40	36-42
Birth weight (gm)						
Mean ± SD	1959 ± 549	3200 ± 376	1644 ± 436	3247 ± 521	3140 ± 302	3200 ± 376
Range	900-2450	2700-4150	920-2400	2700-4200	2800-3600	2700-4150
Placental weight (gm)						
Mean ± SD	437 ± 64	600 ± 120	393 ± 104	627 ± 115	580 ± 144	600 ± 120
Range	310-500	400-700	300-600	400-750	350-700	400-700
Perinatal death	0	0	2	0	0	0

*Gestational age–matched controls.

diagnosed in these women when blood pressure was >160/110 mm Hg at bed rest on at least two occasions 6 hours apart.[13, 14] In these patients proteinuria was >1 gm/L in at least two random specimens collected 6 hours apart.[15] Blood samples were taken before treatment. In 10 of the 16 cases a diagnosis of suspected IUGR was made during the third trimester and confirmed after delivery. In all 10 cases the neonatal ponderal index was <2.20 (1.84 ± 0.25; range, 1.35 to 2.18). In the other six cases birth weight was found to be in the normal range for gestational age, and the ponderal index was >2.20 (2.70 ± 0.32, range, 2.40 to 3.20).

The women with preeclampsia with IUGR differed from the other two groups with respect to gestational age at blood sampling. Therefore this group was compared with a gestational age–matched control group (n = 17). The normotensive pregnancy with IUGR group and the preeclamptic group without IUGR were compared with another gestional age–matched control group (n = 26) (Table I). These two control groups included 43 healthy normotensive women in the third trimester of pregnancy. No complications were observed during pregnancy, and the fetal growth was normal. The women did not receive any type of medication except for a vitamin complex.

The normal nonpregnant control group included 18 healthy nonpregnant women (age, 29.5 ± 6.3 years; range, 20 to 40 years) who did not use oral contraceptives and who were nonsmokers.

Blood samples. Blood samples were obtained by venipuncture in the cubital vein and anticoagulated with 3.8% sodium citrate (9:1, vol/vol, blood/anticoagulant). Citrated blood was centrifuged at 1500 g for 15 minutes at 4° C. Samples were collected and stored at −40° C for ≤3 months before assay.

Informed consent was obtained from all women before the blood sample extraction.

Plasminogen activator inhibitor activity assay. The assay for plasminogen activator inhibitor activity was performed as previously described.[4, 9] Briefly, one volume of sample containing plasminogen activator inhibitor was incubated with single-chain tissue-type plasminogen activator (Biopool, Umea) at a concentration of 40 IU/ml for 15 minutes at room temperature. The reaction was stopped by the addition of an equal volume of 1 mol/L sodium acetate buffer, pH 3.9, and the mixture was incubated at 37° C for 20 minutes to inactivate plasma protease inhibitors. The residual tissue-type plasminogen activator activity was then determined by measuring the plasmin activity generated after addition of plasminogen and soluble fibrin, with the chromogenic substrate S-2251 (Kabi Diagnostica, Stockholm). To obtain optimal assay precision, samples were conveniently diluted so that they inhibited 25% to 75% of the activity of the tissue-type plasminogen activator added. Plasma dilutions were performed with tissue-type plasminogen activator/plasminogen activator inhibitor free plasma obtained by immunoaffinity (Biopool).

One unit of plasminogen activator inhibitor activity is defined as the amount that inhibits 1 IU of single-chain tissue-type plasminogen activator in 15 minutes at room temperature under the conditions used. The intraassay and interassay variability was 6% and 9%, respectively.

Plasminogen activator inhibitor type 1 antigen. Plasminogen activator inhibitor type 1 antigen was

Table II. Fibrinolytic parameters*

	t-PA (ng/ml)	*scu-PA + uPA (ng/ml)*	*PAI activity (U/ml)*	*PAI type 1 antigen (ng/ml)*	*PAI type 2 antigen (ng/ml)*
Nonpregnant controls (n = 18)	4.7 ± 0.01 (3.5-7.0)	0.20 ± 0.08 (0.1-0.3)	1.9 ± 2.1 (0-6)	11 ± 4 (3-22)	<10
IUGR (n = 11)	8.4 ± 4.7 (3.5-20)	0.29 ± 0.10 (0.1-0.5)	23.3 ± 6.9 (7.5-33)	56 ± 29 (16-124)	118 ± 81 (25-290)
Matched controls (n = 26)	9.3 ± 3.5 (4-19)	0.74 ± 0.13 (0.5-0.9)	31.3 ± 11.4 (11-58)	55 ± 19 (24-82)	294 ± 93 (150-550)
p Value	NS	<0.01	NS	NS	<0.0001
Preeclampsia with IUGR (n = 10)	18.1 ± 7.1 (9-31)	0.27 ± 0.18 (0.1-0.6) (n = 6)	45.6 ± 17.7 (21-80)	85 ± 39 (40-140)	58 ± 64 (12-180)
Matched controls (n = 17)	7.5 ± 2.6 (4-13)	0.73 ± 0.17 (0.5-0.9) (n = 8)	26.8 ± 10.9 (12-44)	55 ± 18 (24-82)	319 ± 110 (121-550)
p Value	<0.01	<0.01	<0.05	<0.01	<0.0001
Preeclampsia without IUGR (n = 6)	17.1 ± 13.5 (4.5-40)	0.53 ± 0.36 (0.25-1) (n = 4)	36.3 ± 8.9 (26-48)	106 ± 65 (40-217)	238 ± 49 (200-300)
Matched controls (n = 26)	9.3 ± 3.5 (4-19)	0.74 ± 0.13 (0.5-0.9) (n = 11)	31.3 ± 11.4 (11-58)	55 ± 19 (24-82)	294 ± 93 (150-550)
p Value	<0.05	NS	NS	<0.01	NS
Statistical significance between clinical groups					
IUGR vs PE with IUGR	$p < 0.01$	NS	$p < 0.01$	$p < 0.05$	NS
PE with IUGR vs PE without IUGR	NS	NS	NS	NS	$p < 0.001$

t-PA, Tissue-type plasminogen activator; *scu-PA,* single-chain urokinase-type plasminogen activator; *uPA,* urokinase-type plasminogen activator; *PAI,* plasminogen activator inhibitor; *IUGR,* intrauterine growth retardation; *PE,* preeclampsia.

*Results are given as mean ± SD. Range is shown in parentheses.

quantified with a commercially available, enzyme-linked immunosorbent assay (Tint Elize PAI-1, Biopool). This method detects active and latent (inactive) forms of plasminogen activator inhibitor type 1, tissue-type plasminogen activator/plasminogen activator inhibitor type 1 complexes, and urokinase-type plasminogen activator/plasminogen activator inhibitor type 1 complexes with the same efficiency. The intraassay and interassay variability was 4% and 9%, respectively.

Plasminogen activator inhibitor type 2 antigen. Plasminogen activator inhibitor type 2 antigen was quantified by an enzyme-linked immunosorbent assay (Tint Elize PAI-2, Biopool). The assay detects both the low-molecular-weight (48 kd) form and the high-molecular-weight (60 kd) form of plasminogen activator inhibitor type 2. The intraassay and interassay variability was 3.5% and 6%, respectively.

Tissue-type plasminogen activator antigen. Tissue-type plasminogen activator antigen was determined with a commercially available enzyme-linked immunosorbent assay (Imulyse t-PA, Biopool). The assay detects free and complexed tissue-type plasminogen activator with similar efficiency. The intraassay and interassay variability was 6% and 9%, respectively.

Urokinase-type plasminogen activator and activatable single-chain urokinase activities. Urokinase-type plasminogen activator and activatable single-chain urokinase activities were determined by an immunoabsorbent activity assay (Chromolize u-PA, Biopool). The intraassay and interassay variability was 5% and 10%, respectively.

All these assays include the use of quenching and control antibodies to exclude falsely elevated results. Values represent the mean of duplicate measurements.

Statistical analyses. The Mann-Whitney U and Student t tests and correlation coefficient were used for statistical evaluations.

Results

The clinical data of the groups are shown in Table I. Preeclamptic women with IUGR were delivered earlier than the other groups. Two perinatal deaths were observed in that group.

The fibrinolytic parameters of the different groups are shown in Table II. When the fibrinolytic activators were evaluated, both activators, tissue-type and urokinase-type, increased in the third trimester of normal pregnancy in comparison with the levels observed in normal nonpregnant women (Table II). In relation to tissue-type plasminogen activator antigen levels, an increase was observed in preeclamptic groups with or without IUGR (18.1 ± 7.1 and 17.1 ± 13.5 ng/ml) as compared with normotensive pregnant groups with or without IUGR (8.4 ± 4.7 and 9.3 ± 3.5 ng/ml), respectively (Table II). The levels of the activatable single-chain urokinase plasminogen activator plus active uro-

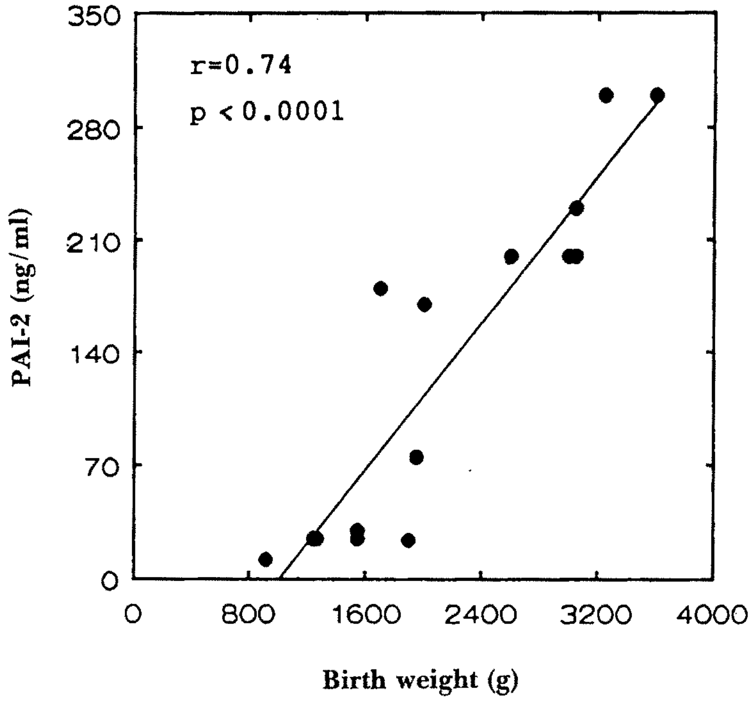

Fig. 1. Correlation between birth weight and plasminogen activator inhibitor type 2 *(PAI-2)* levels in intrauterine growth retardation and preeclampsia.

kinase plasminogen activator activities were lower in IUGR pregnancies, both normotensive and preeclamptic, than in the third trimester of normal pregnancy (matched controls).

The plasminogen activator inhibitor type 1 antigen and activity levels increased significantly in preeclamptic patients, with or without IUGR, in comparison with IUGR normotensive pregnancy or with the group of normal pregnant women (Table II).

In relation to the plasminogen activator inhibitor type 2 antigen levels, a significant decrease in the groups with IUGR was observed (118 ± 81 and 58 ± 64 ng/ml) in comparison with the groups without IUGR (238 ± 49 and 294 ± 93 ng/ml).

When the correlation between birth weight and different parameters was evaluated in the IUGR and preeclamptic groups, a significant correlation ($r = 0.74$, $p < 0.0001$) was observed between plasminogen activator inhibitor type 2 levels and fetal weight (Fig. 1). Moreover, the placental and fetal weight had a good correlation ($r = 0.63$, $p < 0.001$). No significant correlations, however, were obtained between birth weight and the other fibrinolytic parameters studied.

Comment

This study evaluates the fibrinolytic parameters in several groups: normal pregnancy, normotensive pregnancy complicated with IUGR, and preeclampsia with or without IUGR.

With respect to tissue-type plasminogen activator, we confirm previous reports showing that antigen levels increase in severe preeclampsia.[7, 15] Here we show an increase in tissue-type plasminogen activator antigen levels in severe preeclampsia, both with and without IUGR. This increase seems to be related to the activation of the hemostatic system and to the vascular alteration previously reported in preeclampsia.[3, 13, 15-18] In normotensive pregnancy complicated with IUGR, tissue-type plasminogen activator antigen levels are similar to those in matched controls.

The activatable single-chain urokinase plasminogen activator plus urokinase plasminogen activator activities decrease in both normotensive and preeclamptic IUGR groups, but in preeclamptic patients without IUGR no significant differences in the urokinase plasminogen activator levels in comparison with normal pregnancy were obtained. Since urokinase plasminogen activator activity increases during normal pregnancy, as described in a previous report,[19] the decrease in activity in pregnancies with IUGR could suggest that the levels correlate with placental function. However, no significant correlation ($r = 0.40$) was observed between urokinase plasminogen activator levels and fetal weight.

In relation to plasminogen activator inhibitors, an increase in type 1 antigen is observed in the two groups of women with preeclampsia with or without IUGR, in comparison with normal pregnancy. However, a decrease in type 2 antigen levels occurs in preeclampsia with IUGR and in normotensive pregnancies complicated with IUGR. Because plasminogen activator inhibitor type 2 is synthesized by the placenta[20] and localized in villous syncytiotrophoblasts,[21, 22] the decrease in the type 2 level in IUGR pregnancies can be due to the placental alteration observed in these pregnancies.[2, 3, 23]

The placenta is a source not only of type 2 plasminogen activator inhibitor but also of type 1, and type 1 has been found in the invading cytotrophoblasts of human placenta.[22] However, the levels of plasminogen activator inhibitor type 1 are increased in preeclamptic patients but similar in normotensive pregnancies with IUGR in comparison with normal pregnancies. The origin of type 1 can be multiple, from endothelium, activated platelets, or placental vasculature.[22, 24, 25] In preeclamptic patients alterations of endothelium, placenta, and hemostatic system have been described,[3, 13, 15-18] and this may increase, directly or indirectly, the levels of plasminogen activator inhibitor type 1 in severe preeclampsia. On the other hand, the high level of plasminogen activator inhibitor type 1 in severe preeclampsia would contribute to the persistence of fibrin in the uteroplacental vessels.[3]

We conclude that increased type 1 plasminogen activator inhibitor levels are associated with severe preeclampsia and that plasminogen activator inhibitor type 2 levels decrease in pregnancies with IUGR. Plasminogen activator inhibitor type 2 could be considered an adequate marker of placental function.

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Arterial blood flow velocity waveforms of the pelvis and lower extremities in normal and growth-retarded fetuses

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Serial measurements of femoral artery flow velocity waveforms were made at specified intervals of 3 to 4 weeks from 15 to 20 weeks of gestational age to 35 weeks and 1- to 3-week intervals thereafter until delivery in 12 normal fetuses (group 1A) with pulsed Doppler ultrasonography. The pulsatility index of the femoral artery increased linearly with advancing gestation in all 12 fetuses; this was associated with reverse diastolic flow velocity waveforms. In 30 other normal fetuses (group 1B) whose gestational ages ranged between 16 and 38 weeks (mean ± SD, 29.5 ± 5.6 weeks), the femoral artery flow velocity waveforms were analyzed with the external iliac artery, internal iliac artery, and umbilical artery waveforms. No significant difference was observed between the pulsatility index of the external iliac artery and that of the femoral artery (mean ± SD, 3.7 ± 1.1 vs 3.7 ± 1.1). The pulsatility index of the internal iliac artery was significantly higher than that observed in the umbilical artery (mean ± SD, 1.27 ± 0.28 vs 1.12 ± 0.23). The femoral and umbilical artery velocity waveforms also were determined in 20 fetuses with intrauterine growth retardation (group 2), whose gestational ages ranged between 24 and 36 weeks (mean ± SD, 31 ± 3.4 weeks). Abnormal femoral artery pulsatility index was recorded in two fetuses who died. Absent or reverse flow of the umbilical artery was recorded in 10 fetuses; four of them died, and four others manifested fetal distress or required admission to the neonatal intensive care unit or both. These preliminary data suggest that the femoral artery pulsatility index cannot be used as an indicator of adverse fetal outcome, whereas absent or reverse flow of the umbilical artery seems to be better correlated with adverse fetal outcome. (AM J OBSTET GYNECOL 1991;165:143-51.)

Key words: Femoral artery velocimetry, external iliac artery velocimetry, internal iliac artery velocimetry, pulsatility index, IUGR

Much of our knowledge of the circulation of the human fetus is derived from animal work, which has shown that distribution of the cardiac output to the kidneys is 1.8% to 2.9% during gestation, whereas the total carcass of the fetus receives a mean of 32% to 40% of the combined ventricular output; 16% is distributed to the lower carcass and 15% to 23% to the upper carcass.[1] It has been shown that there is a redistribution of the blood flow from the periphery to the brain during fetal hypoxemia in the lamb.[2] Therefore the peripheral circulation in the skin and muscles, which receives a large percentage of fetal cardiac output, is the site where vasomotor responses may affect major redistribution of the fetal circulation.

Pulsed Doppler ultrasonography represents a noninvasive method that allows examination of the cardiovascular system of the human fetus. Recently, combined Doppler studies have reported that hypoxemia in the fetal lamb is associated with abnormal femoral artery

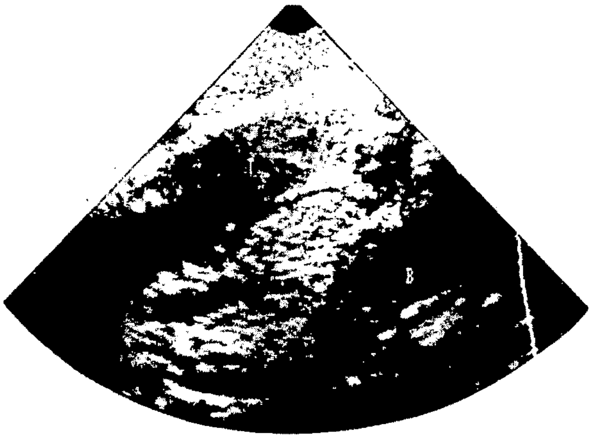

Fig. 1. Axial section of bladder *(B)* and thigh *(T)* showing relationship between fetal external iliac artery *(EI)* inside pelvis and femoral artery *(FA)* outside pelvis in normal fetus at 28 weeks' gestation.

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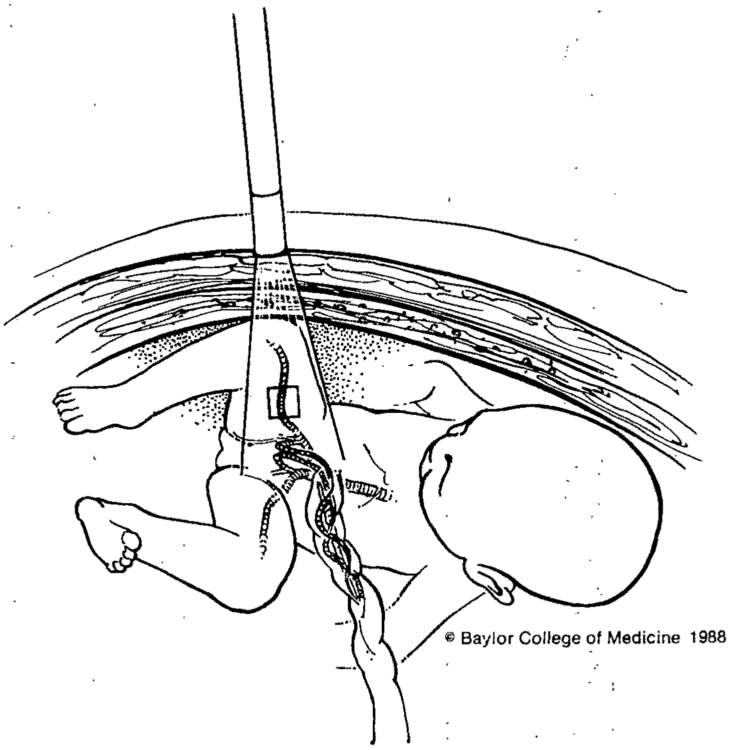

Fig. 2. Drawing showing ideal plane used in Doppler ultrasonographic studies of femoral artery.

flow velocity waveforms without appreciable effect on umbilical artery velocity waveforms.[3] Thus the study of the femoral artery with Doppler ultrasonography can provide information regarding the vascular resistance of the lower extremities and can also contribute to our understanding of the fetal circulation in normal fetuses and fetuses with intrauterine growth retardation (IUGR).

This communication introduces a pulsed Doppler method for recording flow velocity waveforms from the femoral artery, and the external and internal iliac arteries. The data obtained in 12 fetuses who were followed up longitudinally have permitted an evaluation of the variability of the femoral artery velocity waveforms during gestation and have provided the basis for the development of tables giving the normal variability as a function of gestational age. Furthermore, femoral and umbilical artery flow velocity waveforms obtained in 20 fetuses with IUGR are reported and compared with those obtained in normal fetuses.

Material and methods

This study was carried out with data collected from 42 normal fetuses (group 1) and 20 fetuses with IUGR (group 2) between May 1988 and February 1990. The patients of the two groups were primarily middle-class women from the Houston area. The patients of the first group were delivered of infants after 37 weeks' gestational age, and comprehensive pediatric assessment within 24 hours of delivery revealed no evidence of growth abnormalities or congenital anomalies. Birth characteristics such as weight, length, head circumference, and abdominal circumference were within the normal ranges for singletons determined by Usher and McLean.[4]

Fetal age determination. Fetal age determination for the fetuses of group 1 was based on the last menstrual period, which was confirmed by first-trimester and early second-trimester ultrasonographic examinations (crown-rump length, biparietal diameter, and femur diaphysis length measurement) in all of the fetuses. For the fetuses of group 2 gestational age was determined by both menstrual history and ultrasonographic assessment of fetal biometry either from a scan before 20 weeks' gestation or, if such a scan had not been performed, from the first ultrasonographic assessment in this study. Gestational age was based on the last menstrual period when it differed by <2 weeks from that determined by ultrasonographic parameters. Gestational age was based on the ultrasonographic assessment if the patient was unsure of her last menstrual period or if the discrepancy between last menstrual period and ultrasonographic gestational age was >2 weeks.

Fetal growth retardation was defined on the basis of ultrasonographic measurements of head and abdominal circumference >2 SD below the normal mean value for gestational age,[5] birth weight >2 SD below the normal mean value for gestational age,[4] decreased amniotic fluid volume,[6] and malnourished infant.[7] When three of these criteria were satisfied, the infant was categorized as having IUGR. The data obtained from the Doppler studies were not used in the clinical management of the patients.

Doppler examinations. Doppler examinations of the femoral artery in 12 fetuses of group 1 (group 1, A) were carried out at 3- to 4-week intervals from 15 to 20 weeks' gestational age (initial scan, mean ± SD, 17 ± 1.8 weeks) to 35 weeks and at 1- to 3-week intervals thereafter until delivery. To determine whether different arteries of the pelvis and lower extremities had a different pulsatility index value, the femoral artery flow velocity waveforms were analyzed with the external and internal iliac arteries and the umbilical artery on one occasion in 30 other normal fetuses between 16 and 38 weeks' gestation (mean ± SD, 29.5 ± 5.6 weeks). In the fetuses with IUGR (group 2) whose gestational ages ranged between 24 and 36 weeks (mean ± SD, 31 ± 3.4 weeks), the femoral artery and the umbilical artery were studied on one occasion.

A General Electric PASS II (General Electric Medical Systems, Milwaukee) dynamic image scanner was used. The 2.5 MHz transducer used a 2 mm sample volume. A pass filter between 0 and 50 Hz was used. Doppler ultrasonographic energy was 114 mW/cm^2 spatial peak temporal average intensity. The fetuses were examined with the mothers in a semirecumbent position. Doppler examination of the femoral artery was performed in the following sequence: After imaging of a longitudinal section of the thigh and pelvis in which the bladder could be visualized (Fig. 1), the sample volume was positioned on the femoral artery at the upper third of the thigh (Fig. 2), and Doppler waveforms of the femoral artery and vein were displayed on either side of the baseline (Fig. 3). When the femoral artery could not be visualized clearly, as shown in Fig. 1, the sample volume was positioned inside the pelvis, along the side of the bladder, where two different waveforms could be recorded at the same time, as reported previously.[8] This point corresponded to the bifurcation of the common iliac artery into the internal and external iliac arteries, as has been determined by following the common iliac artery to this point. The waveform at higher diastole corresponded to the internal iliac artery from which the umbilical artery takes its origin, whereas the waveforms at low diastole corresponded to the external iliac artery.[8] From the point at which the two waveforms were imaged, the sample volume was moved toward the thigh, which enabled the signal to be recorded at different levels along the external iliac artery until it left the pelvis and became the common femoral artery.

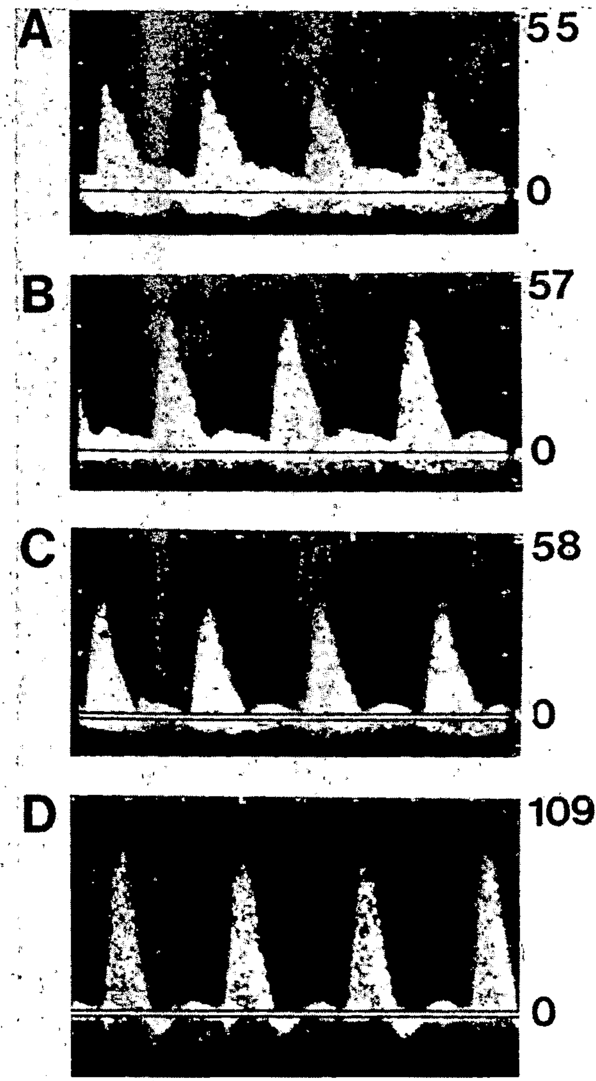

Fig. 3. Flow velocity waveforms from fetal femoral artery at 18 (A), 24 (B), 30 (C), and 39 weeks' gestation with forward diastolic flow in A, presence of notch in B and C, and reverse flow in D.

In 10 normal fetuses, two-dimensional directed pulsed Doppler waveforms (Acuson 128, Acuson Co., Mountain View, Calif.) were compared with pulsed Doppler waveforms with color-flow Doppler imaging (Acuson 128). Before the femoral artery was interrogated with pulsed Doppler ultrasonography, color-flow mapping was used to confirm that the sample volume had been positioned on the femoral artery.[8] Color Doppler was also used to distinguish anatomically the external and internal iliac arteries.

Data analysis. The Doppler velocity waveforms were recorded on videotape, and a digitized computer system (Digisonics Inc., Houston) was used to calculate the following variables: peak velocity, lowest diastolic velocity, and mean velocity. Three consecutive wave-

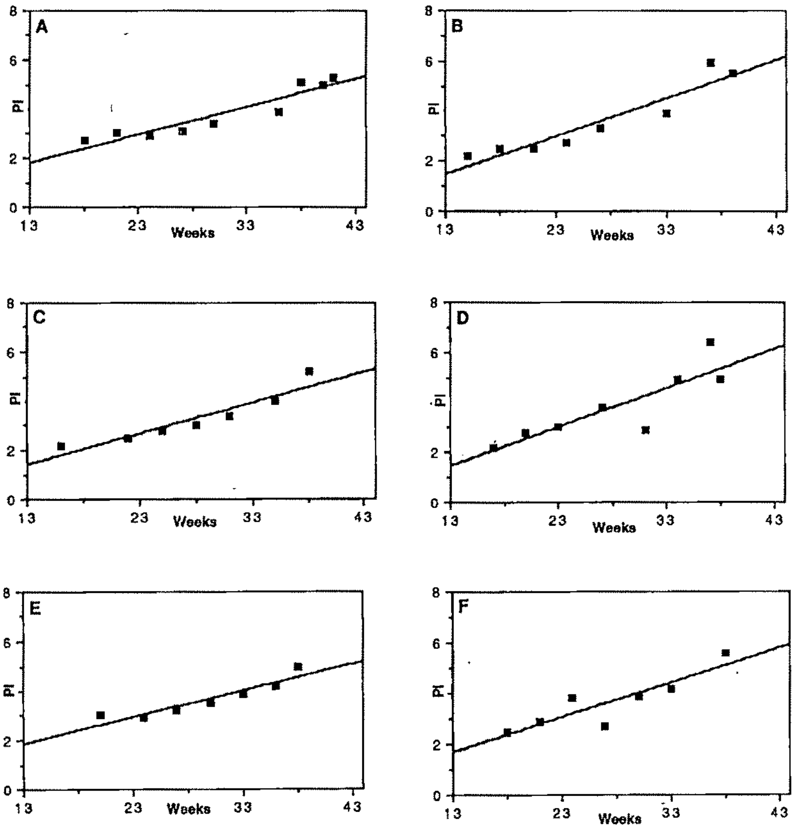

Fig. 4, ***A*** to ***F*.** Individual femoral artery pulsatility index values of six normal fetuses during gestation as determined from serial measurement.

forms were analyzed, and the results were averaged. The pulsatility index[9] [(Peak velocity − Lowest diastolic velocity)/Mean velocity] was then calculated. Measurements were obtained only during fetal apnea, which was defined as the absence of visible changes in the waveform patterns for at least 15 waveforms.

The intraobserver error (coefficient of variation) for the pulsatility index of the femoral artery was 7.7%, as has been reported previously.[8]

Changes in pulsatility index with gestational age for the fetuses of group 1, A, were characterized quantitatively by fitting mathematical functions to the experimental data. For each fetus regression analysis was used to compare the pulsatility index with gestational age. We looked for the optimal polynomial model on the basis of two criteria: coefficients different from zero and maximal coefficient of determination (R^2). Analysis of variance was used to determine if the regression accounted for a significant part of the variability in the dependent variable. A $p < 0.05$ was considered to indicate statistical significance. Corresponding coefficients for individual fetuses, as well as the R^2 values, were averaged, and their standard deviations were determined and used to construct tables giving the normal variability as a function of gestational age.

In the fetuses of group 1, B, analysis of variance and a multiple comparison test (Newman-Keuls test) were used for statistical analysis. A $p < 0.05$ was considered to indicate statistical significance.

The pulsatility index values for the IUGR fetuses (group 2) were compared with those of the normal fetuses (group 1, A). All analyses were performed on a Macintosh Plus computer with the Stat View statistical package (Albacus Concepts Inc., Berkeley, Calif.).

Results

The patients of group 1, A, were delivered of fetuses between 38 and 42 weeks' gestation (mean ± SD, 39.7 ± 1.2 weeks). Fetal femoral artery flow velocity waveforms in these fetuses were successfully obtained from 93 of the 106 examinations attempted (88%). Failure to obtain flow velocity waveforms was the result of fetal movements (especially before 30 weeks' gestation) and fetal breathing.

The pulsatility index increased linearly during gestation in all of the fetuses of group 1, A (Fig. 4, *A* to *F*, and Fig. 4, *G* to *L*); this was consistent with the presence of reverse flow in the diastolic component of the femoral artery waveform (Fig. 3) in the third trimester in those fetuses followed up longitudinally.

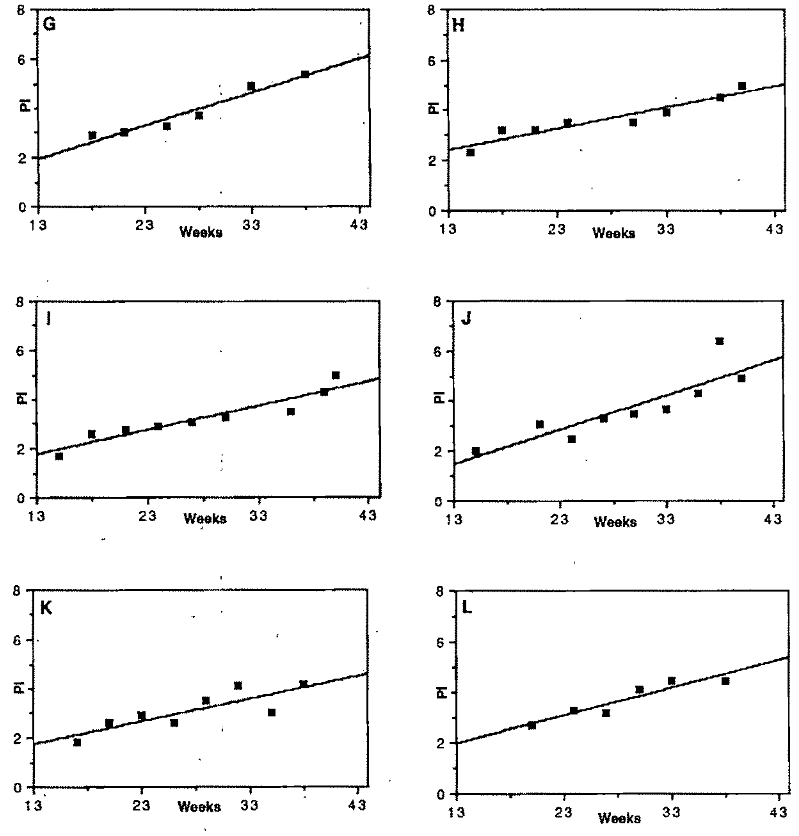

Fig. 4, *G* to *L*. Individual femoral artery pulsatility index values of six normal fetuses during gestation as determined from serial measurement.

Table I. Linear model of individual femoral artery pulsatility index

No.	a_0	*Pulsatility index* = a_0 + a_1 *(gestational age)*			
		Coefficient a_0 *different from 0*	a_1	*Coefficient* a_1 *different from 0*	R^2
1	0.327	No	0.114	Yes	87
2	−0.504	No	0.152	Yes	87
3	−0.210	No	0.126	Yes	85
4	−0.546	No	0.155	Yes	70
5	0.477	No	0.107	Yes	83
6	−0.077	No	0.137	Yes	74
7	0.171	No	0.136	Yes	93
8	1.308	No	0.085	Yes	88
9	0.499	Yes	0.099	Yes	86
10	−0.306	No	0.138	Yes	72
11	0.545	No	0.092	Yes	65
12	0.560	No	0.110	Yes	85

The table presents coefficients (a_0 and a_1) of linear model fitted to femoral artery pulsatility index measurements obtained serially at different gestational ages in 12 fetuses. R^2, Coefficient of determination.

Table I presents the coefficients of the linear model and the R^2 for each individual fetus. The changes in pulsatility index as a function of gestational age are presented in Table II. Analysis of variance indicated that the relationship to gestational age was statistically significant in these fetuses.

In nine fetuses followed up longitudinally, who were not included in our analysis because of preterm labor (n = 1), first pulsatility index measurement after 20 weeks' gestation (n = 5), hypertension (n = 2), or multicystic kidney (n = 1), the pulsatility index increased linearly and was associated with the presence of reverse

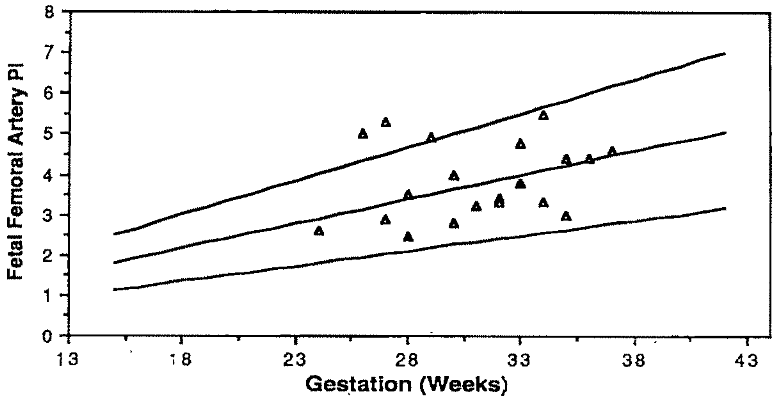

Fig. 5. Reference range (mean ± 2 SD) of femoral artery pulsatility index with gestation constructed from study of 12 normal fetuses followed up longitudinally. *Triangles* represent pulsatility index values of 20 fetuses with IUGR.

Table II. Fetal femoral artery pulsatility index: normal values

Gestational age (wk)	*Normal value*		*Predicted value*	*Normal value*	
	−2 SD	*−1 SD*		*+1 SD*	*+2 SD*
15	1.1	1.4	1.8	2.1	2.5
16	1.2	1.5	1.9	2.3	2.6
17	1.2	1.6	2.0	2.4	2.8
18	1.3	1.7	2.1	2.5	3.0
19	1.4	1.8	2.2	2.7	3.1
20	1.5	1.9	2.4	2.8	3.3
21	1.5	2.0	2.5	3.0	3.5
22	1.6	2.1	2.6	3.1	3.6
23	1.7	2.2	2.7	3.3	3.8
24	1.8	2.3	2.9	3.4	4.0
25	1.8	2.4	3.0	3.6	4.1
26	1.9	2.5	3.1	3.7	4.3
27	2.0	2.6	3.2	3.8	4.5
28	2.1	2.7	3.3	4.0	4.6
29	2.1	2.8	3.5	4.1	4.8
30	2.2	2.9	3.6	4.3	5.0
31	2.3	3.0	3.7	4.4	5.1
32	2.4	3.1	3.8	4.6	5.3
33	2.4	3.2	3.9	4.7	5.5
34	2.5	3.3	4.1	4.8	5.6
35	2.6	3.4	4.2	5.0	5.8
36	2.7	3.5	4.3	5.1	6.0
37	2.7	3.6	4.4	5.3	6.1
38	2.8	3.7	4.5	5.4	6.3
39	2.9	3.8	4.7	5.6	6.5
40	3.0	3.9	4.8	5.7	6.6
41	3.0	4.0	4.9	5.9	6.8
42	3.1	4.1	5.0	6.0	7.0

Pulsatility index = 0.121(gestational age) (R^2 = 81 ± 9). 1 SD = 0.023.

flow in the diastolic component of the femoral artery with advancing gestation.

In fetuses of group 1, B, analysis of variance indicated significant differences in the pulsatility index values of the four vessels studied (F = 91.327, $p < 0.0001$). The Newman-Keuls test revealed a significantly higher value for the pulsatility index in the internal iliac artery than in the umbilical artery (1.27 ± 0.28 vs 1.12 ± 0.23). The external iliac artery and the femoral artery did not show any significant difference (3.7 ± 1.1 vs 3.7 ± 1.1), and the external and femoral arteries showed a significantly higher value when compared with that of both the umbilical artery and the internal iliac artery.

The femoral artery pulsatility index of two fetuses with IUGR was higher than 2 SD for gestational age (Fig. 5). The umbilical artery pulsatility index was abnormal in 10 fetuses (with absent or reverse diastolic

Table III. Clinical details in 20 growth-retarded fetuses at time of test

No.	*Gestational age (wk)*	*Hypertension**	*Proteinuria†*	*Pulsatility index*	
				Femoral artery	*Umbilical artery*
1	24	+	+	2.6	1.9 (RF)‡
2	26	–	–	5.0‡	4.2 (RF)‡
3	27	–	–	5.3‡	0.8
4	28	+	+	3.5	1.8 (AF)‡
5	28	–	–	2.4	3.1 (RF)‡
6	28	–	–	2.9	1.7 (AF)‡
7	29	+	+	4.9	1.1
8	30	–	–	3.8	0.9
9	30	–	–	2.8	2.1 (AF)‡
10	31	–	–	3.2	0.9
11	32	+	–	3.3	1.2
12	32	–	–	3.4	1.0
13	33	+	+	4.8	2.0 (AF)‡
14	33	–	–	3.8	2.8 (RF)‡
15	34	–	–	5.5	1.1
16	34	+	–	3.3	1.8 (AF)‡
17	35	–	–	3.6	1.9 (AF)‡
18	35	–	–	3.9	1.0
19	35	–	–	3.0	0.7
20	36	–	–	4.6	1.0

RF, Reverse diastolic flow; *AF,* absent end-diastolic flow.
*Diastolic blood pressure >90 mm Hg on two or more occasions separated by >24 hours.
†Proteinuria >0.5 gm/L on more than one occasion.
‡Values above estimated upper limit for gestational age.

flow velocity waveform); four of them died, and in four other fetuses either fetal distress developed or they were admitted to the neonatal intensive care unit or both (Tables III and IV).

Comment

A study by Stewart et al.[10] assessed the maximum velocity waveform in the external iliac artery. These authors stated that during normal pregnancy there is continuous forward flow throughout the cardiac cycle in the external iliac artery; this is associated with a decrease in pulsatility index of this artery. The same authors demonstrated that in fetuses with IUGR the pulsatility index of the external iliac artery increases, which suggests a redistribution of the blood flow from the lower extremities to the brain. These authors observed that the femoral artery would have been a more obvious choice for assessing lower-extremity blood flow. However, despite color-coded Doppler, they only rarely succeeded in identifying this vessel because of the related course of the femoral artery and femur, which often concealed the vessel from ultrasonographic inspection. In addition, they reported that the external and internal iliac arteries can be easily identified by color-flow mapping.

Our data show that flow velocity waveforms of the fetal femoral artery and the external and internal iliac arteries can be obtained by the pulsed Doppler system and color-flow mapping. In our experience it is easy to use color Doppler to visualize the internal iliac artery in all of the cases; however, it is more difficult to visualize the external iliac artery and the femoral artery in all of the cases. There are two reasons for this. The first is anatomic. In the fetus the internal iliac artery is twice as large as the external iliac artery.[11] The second reason concerns the characteristic waveforms. The internal iliac artery presents, as does the umbilical artery, continuous diastolic flow, whereas the external iliac artery and the femoral artery have low or reverse diastolic flow, which indicates intermittent flow toward the thigh and leg. With color Doppler it is possible to follow the internal iliac artery, which is positioned along the side of the bladder. This artery is directed downward; then it becomes anterior to the bladder and runs superiorly on the inner aspect of the anterior wall of the abdomen to reach the umbilicus and converges toward the same vessel from the opposite side. Having passed through the umbilical opening, the two arteries, which are now called *umbilical arteries*, enter the umbilical cord, become coiled around the umbilical vein, and ultimately ramify in the placenta.[11] Through the internal iliac artery passes the blood flow that is directed to the placenta, whereas the blood flow that is directed mainly to the lower extremities passes through the external iliac artery.

We have done a longitudinal study of the femoral artery velocity waveforms, and we found femoral artery reverse flow waveforms late during gestation in all of

Table IV. Clinical details of 20 growth-retarded fetuses at delivery

No.	Gestation at delivery (wk)	Mode of delivery	Apgar score		Umbilical vein pH	Birth weight (gm)	Presently alive
			1 min	5 min			
1	26	C/S*,†	5	9	7.21	580‡	No
2	29	IVD§	0	0	–	380‡	No
3	28	C/S†	9	10	7.28	910	No
4	36	C/S*	7	9	–	1800‡	Yes
5	28.5	C/S*,†	6	8	7.25	750‡	Yes
6	28	C/S*	8	9	7.30	480‡	No
7	29	C/S*,†	9	9	7.38	960	Yes
8	36	SVD	9	9	–	1500‡	Yes
9	34	C/S	8	9	7.32	1250‡	Yes
10	38	SVD	8	9	–	2095‡	Yes
11	32.5	C/S	9	9	7.30	1500	Yes
12	36	C/S*	7	9	7.19	2610	Yes
13	34	C/S†	8	9	–	1080‡	Yes
14	34	IVD	8	9	7.28	1800	Yes
15	34	SVD	9	10	7.29	2020	Yes
16	34	C/S*	9	9	7.35	1400‡	Yes
17	35.5	C/S*,†	5	7	–	1350‡	No
18	36	SVD	8	9	–	1800‡	Yes
19	42	SVD	9	9	–	2400‡	Yes
20	37	SVD	8	9	–	1900‡	Yes

C/S, Cesarean section; *SVD*, spontaneous vaginal delivery; *IVD*, induced vaginal delivery.
*Abnormal fetal heart rate.
†Admission to neonatal intensive care unit.
‡Values >2 SD below mean birth weight for gestational age.
§Fetal death.

the fetuses, as well as an increase of the pulsatility index of the fetal femoral artery with advancing gestation. We have not found a significant difference between the pulsatility index values of the external iliac artery and the femoral artery. When the vascular anatomy of the pelvis and thigh is considered, it is easy to understand why there is no difference in the waveforms of these two arteries. The external iliac artery extends from the bifurcation of the common iliac artery to a point beneath the inguinal ligament, midway between the anterior superior spine of the ilium and the symphysis pubis. Entering the thigh below the inguinal ligament, the vessel becomes the femoral artery. The external iliac artery gives off few branches, the largest two of which are the inferior epigastric and the deep iliac circumflex.[11] The femoral artery and the external iliac artery differ only in location (inside the pelvis, external iliac artery; outside the pelvis, femoral artery).

Our results are the opposite of those reported by Stewart et al.[10] The pulsatility index values of the external iliac artery and the umbilical artery reported by those authors are quite similar. The only difference between the velocity waveforms of these two arteries is the higher pulsatility index value of the external iliac artery. Our study shows that the pulsatility index of the internal iliac artery is higher than that of the umbilical artery. This difference can be compared with the different umbilical artery pulsatility index values obtained at the level of the placenta and at the level of abdominal insertion.[12] The waveforms of the external iliac artery and internal iliac artery are completely different.[8] We have never seen external iliac artery waveforms such as those presented by Stewart et al.[10] The waveforms presented by those authors are similar to those of the umbilical artery and internal iliac artery.

The reverse flow, which appears in the third trimester in the fetal femoral artery flow velocity waveform, could be due to an increase of the resistance in the lower extremities, as Rittenhouse et al.[13] have shown in animal experiments. The blood flow at the level of the common iliac artery finds a high resistance in the external iliac artery, and therefore it is mainly directed toward the placenta; this is known to decrease the vascular resistance with advancing gestation.[14-16]

Our study shows that in 2 fetuses with IUGR, the pulsatility index of the fetal femoral artery was above the upper limit for gestational age. The two fetuses died; the first in utero, the second after 23 days of life. The femoral artery pulsatility index was normal in three other fetuses who died after delivery and in six other fetuses in whom fetal distress developed in labor or who were admitted to the neonatal intensive care unit or both. The absent or reverse diastolic velocity of the umbilical artery was associated with four fetal deaths and with four fetuses in whom fetal distress developed or who were admitted to the neonatal intensive care unit or both.

The clinical importance of our results is that reverse flow of the fetal femoral artery and external iliac artery is normal in the third trimester and is not associated with fetuses with IUGR and poor fetal outcome, as has been reported for the umbilical artery.[14] In addition, the study of the femoral artery does not seem to be a good indicator of adverse fetal outcome when compared with study of the umbilical artery, in which a correlation between abnormal waveforms and poor placental gas exchange has been reported.[17] Furthermore, more extensive studies are necessary.

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The influence of contamination of culture medium with hepatitis B virus on the outcome of in vitro fertilization pregnancies

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Heat-inactivated human serum is added to the culture medium used for in vitro fertilization and other forms of assisted conception. Because one batch of pooled serum contained hepatitis B virus, an epidemic occurred among women participating in the treatment program. Seventy-nine women had serologic proof of hepatitis B infection. This incident gave the opportunity to study the effect of hepatitis B virus on pregnancy outcome and the newborn. The situation is unique because the preimplantation embryo was exposed to hepatitis B virus or the pregnancy was complicated by a (sub)clinical infection. Twenty-four women were or became pregnant while having an acute hepatitis B infection. Five pregnancies ended in abortion. The remaining 19 pregnancies ended in the birth of 24 children. No evidence for any harmful effect of exposure to hepatitis B virus in the embryonic or fetal period on the newborn could be found. (AM J OBSTET GYNECOL 1991;165:152-9.)

Key words: In vitro fertilization, hepatitis B infection, pregnancy outcome

Hepatitis B epidemics have occurred from time to time as a result of medical procedures such as vaccination,[1,2] acupuncture,[3] surgery,[4-7] or administration of red blood cells to elicite erythrocyte antibodies.[8] On March 2, 1988, a hepatitis B epidemic was discovered in women treated at our in vitro fertilization (IVF) center during the preceding months. During the IVF procedure, oocytes, spermatozoa, and, after fertilization, preembryos are kept in tissue culture medium containing 7.5% heat-inactivated human serum. The human serum for the tissue culture medium is usually obtained from the woman involved, but in special cases pooled serum from pregnant women is used. Because culture medium with pooled serum appeared to improve the results of IVF, pooled serum was also used in cases without specific indication for this approach.

In this study we describe a hepatitis B epidemic caused by a human serum pool contaminated with hepatitis B virus. The contaminated culture medium had been used from Nov. 2, 1987, until Jan. 21, 1988. In this period 175 couples (193 treatments) had been treated with IVF and embryo transfer, transfer of frozen-thawed preembryos or other forms of assisted conception (i.e., intrauterine insemination, direct intraperitoneal insemination, and gamete intrafallopian transfer). For transfer of frozen-thawed preembryos and for intrauterine insemination, direct intraperitoneal insemination, and gamete intrafallopian transfer treatment, only culture medium with 7.5% (vol/vol) pooled serum was used. The inoculation volume was about 50 μl of culture medium.

The effect of the hepatitis B infection on the course of pregnancy and the unborn child is described in this article.

Patients, pregnancies, and treatment

All couples involved were instructed to come as soon as possible to the outpatient department for blood sampling and passive and active immunization with anti-hepatitis B immunoglobulin (HBIG) (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam) and HBvax-DNA (Merck Sharp & Dohme, West Point, Pa.). All serum samples were tested for hepatitis B surface antigen (HBsAg) and for antibodies to hepatitis B core antigen by enzyme-linked immunosorbent assay. If these were present, supplementary tests for hepatitis B e antigen (HBeAg) and for antibodies to HBsAg (anti-HBs) and HBeAg (anti-HBe) were performed. The test results of this first screening indicated that the original period could be divided into two parts: Nov. 2 until Dec. 13, 1987 (period A)—use of culture medium containing hepatitis B virus (HBV) certain; Dec. 13, 1987, until Jan. 21,

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1988 (period B)—use of culture medium containing HBV improbable, because none of the women treated in this period had signs of a hepatitis B infection (acute hepatitis B infection, seroconversion, or the presence of anti-HBc antibodies of the immunoglobulin M type. Unfortunately, we could not examine the original serum because all of it had been used for preparing the culture medium. Also, all of the contaminated culture medium was used, so we could not check that either.

An overview of all kinds of treatment is given in Table I.

The 166 IVF treatments resulted in 40 clinical pregnancies (a positive urine pregnancy test, detection level 50 IU/L, 17 days after ovum pickup). Nine of these pregnancies ended in clinical abortion between 7 and 14 weeks of amenorrhea; there was one ectopic pregnancy. Eight women who had a treatment not resulting in pregnancy in period A or B became pregnant after Jan. 21, 1988 (seven of these pregnancies were the result of IVF treatment; one woman conceived spontaneously). One of those seven IVF pregnancies ended in clinical abortion. Transfer of cryopreserved preembryos resulted in two clinical pregnancies, both of which ended in a clinical abortion. The intrauterine insemination, direct intraperitoneal insemination, and gamete intrafallopian transfer treatments did not result in pregnancy.

Table II shows the number of clinical IVF pregnancies and pregnancy outcome in periods A and B and the outcome of the above-mentioned seven pregnancies that occurred after Jan. 21, 1988, compared with a control period (January to October 1987). With the χ^2 test no significant differences in the percentage of pregnancy per embryo transfer ($p = 0.8$), percentage of abortion ($p = 0.8$), and percentage of multiple pregnancy ($p = 0.9$) could be found.

In period A the culture medium was prepared with pooled serum containing HBV. The preembryo was exposed to HBV by means of this "pool culture medium." The developing fetus could be exposed to the HBV if the mother suffered from a (sub)clinical hepatitis B infection as a result of contamination with the HBV at embryo transfer. In period B the pool culture medium probably was free of HBV. One of the women who conceived in this period also had been treated with IVF and embryo transfer in period A. She was still HBsAg negative at the time of the IVF treatment that resulted in the pregnancy, but was HBsAg positive for a short period of time during the first trimester (<13 weeks), so this fetus from period B could have been exposed to HBV at this later age. Of the eight women who were infected with HBV during IVF treatment in period A but became pregnant after Jan. 21, 1988, six were HBsAg positive at the time of conception, whereas two were already in the convalescent phase. The preembryos of the six women who were HBsAg positive at the time of conception were exposed to HBV when they were cultured in medium with 7.5% patient serum, while the developing fetus could be exposed to HBV because of the viremia of the mother.

Table I. Subdivision of 193 treatments performed in period A and period B

	Period A	*Period B*	*Total*
IVF and embryo transfer	116	50	166
Transfer of preembryos cryopreserved before Nov. 2, 1987	10	1	11
Transfer of preembryos cryopreserved between Nov. 2 and Dec. 13, 1987		2	2
Cryopreservation of preembryos*	8	1	9
Direct intraperitoneal insemination	1	2	3
Intrauterine insemination	1		1
Gamete intrafallopian transfer	1		1
TOTAL	137	56	193

*These preembryos were thawed and transferred after Jan. 21, 1988, but were potentially HBV contaminated by the pooled serum from period A or B.

At the first screening a blood sample was taken from all couples treated in the above-mentioned period and passive and active immunization (5 ml HBIG and 10 µg HBvax-DNA) administered to them. The results of the blood test (enzyme-linked immunosorbent assay) were known the next day. If the test showed serologic proof of a (cured) hepatitis B infection, the immunization was discontinued. The full immunization scheme consisted of 5 ml of HBIG in months 0 and 1 and 10 µg of HBvax-DNA in months 0, 1, 2, and 6.

To avoid needless immunization of pregnant women, their blood was checked with a rapid HBsAg test (Hepatest, Wellcome Laboratories, Dartford, England). If the test result was negative, the woman was immunized (Fig. 1). When the first screening was performed, 39 women knew they were pregnant; the duration of pregnancy varied between 4 and 19 weeks. Seven of them were slightly or severely icteric. With the rapid HBsAg test, 12 of the 39 pregnant women were found to be HBsAg positive, while the enzyme-linked immunosorbent assay showed that 14 of them were HBsAg positive (rapid HBsAg test = 12/14, 86% true positive; 2/14, 14% false negative). The two women who had a false-negative rapid HBsAg test had a very low titer of HBsAg in the enzyme-linked immunosorbent assay.

After the first screening two women were discovered to be pregnant. One of these pregnancies was the result of IVF treatment with embryo transfer on Feb. 28,

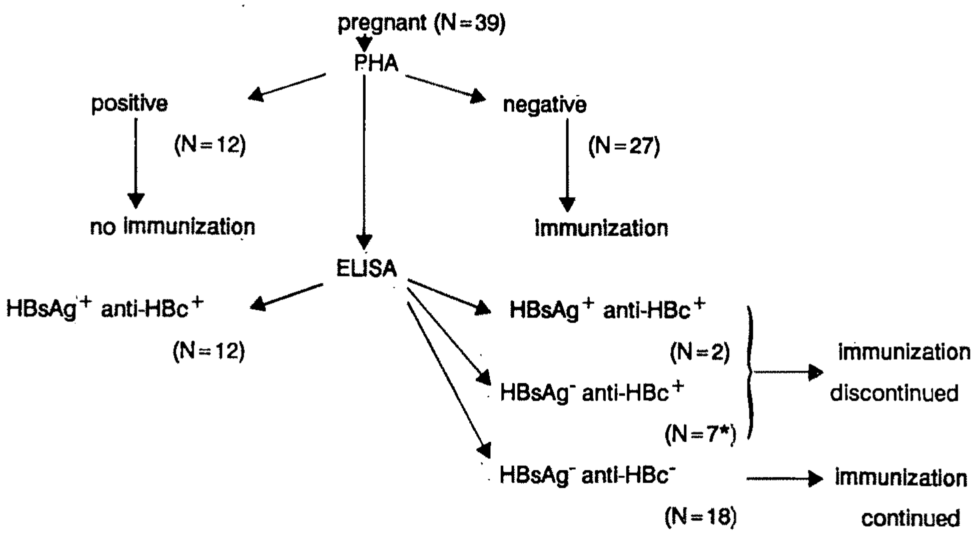

Fig. 1. Management of women who were pregnant at time of first screening. Immunization was started only if a rapid HBsAg test was negative. If enzyme-linked immunosorbent assay showed serologic proof of a (cured) hepatitis B infection, immunization was discontinued. *Asterisk,* One woman with positive preserum, serologic proof that she had recovered from past hepatitis B infection before IVF was started.

Table II. Total number of IVF pregnancies, percentage of clinical pregnancy per embryo transfer, number and percentage of abortions (including ectopic pregnancy), and multiple pregnancies in period A, in period B, after Jan. 21, 1988, and in a control period (January to October 1987)

	Total No. of IVF pregnancies	*Percentage of pregnancy per embryo transfer*	*Abortion*		*Multiple pregnancy**	
			No.	*%*	*No.*	*%*
Period A	26	22	7	27	5	26
Period B	14	28	3	21	2	18
After Jan. 21, 1988	7	23	1	14	2	33
Jan.-Oct. 1987	80	23	21	26	14	23

χ^2 Test: For percentage of pregnancy per embryo transfer, $p = 0.8$; for percentage of abortion, $p = 0.8$; for percentage of multiple pregnancy, $p = 0.9$ (no significant differences).

*Percentage of multiple pregnancies in all ongoing pregnancies.

1988, whereas the other one occurred spontaneously. Four pregnancies ended in clinical abortion after the first screening. Two of them were viable pregnancies (fetal heartbeats had been visualized with ultrasonography) and the remaining were blighted ova.

Table III represents the relationship between pregnancy outcome and the immunization scheme followed.

The course of pregnancy in women with and without serologic signs of HBV infection is shown in Table IV. No significant differences for percentage abortions ($p = 0.5$) or percentage multiple pregnancy ($p = 0.9$) could be found with the Fisher exact test.

According to the amount of HBV that was introduced into the uterine cavity three categories could be distinguished: category 1, the preembryo was cultured in the contaminated medium and about 50 μl of contaminated medium was used for transfer of the preembryos into the uterine cavity; category 2, the preembryos were cultured in the contaminated medium but were transferred into the uterine cavity in medium free of HBV; category 3, the contaminated medium was used for neither embryo culture nor embryo transfer, and the only contact with the HBV was by means of the glass capillary used to remove the cumulus cells from the oocyte. Of the couples treated with IVF between Nov. 2 and Dec. 13 (period A) only 86 could be classified with certainty into one of these categories.

In Table V the number and percentage of pregnancies in each category are shown. The figures indicate that contact with HBV during IVF treatment had no influence on the pregnancy rate or the course of pregnancy. There are no significant differences, except for the percentage of HBV infections (χ^2 test for trend, $p < 0.001$).

Table III. Potentially HBV-infected IVF pregnancies and administration of HBIG and HBvax-DNA

	Total	*No immunization*	*HBIG and HBvax-DNA once*	*Full scheme*
Pregnant at time of first screening	39	12	9*	18
Pregnancy discovered after first screening	2	1	1	—
Abortion after first screening	4	—	1	3
Pregnancies resulting in birth of living children	37	13	9	15

*One woman with positive preserum.

Table IV. Total number of clinical pregnancies and number and percentage of abortions and multiple pregnancies in women with and without serologic signs of HBV infection

	Total No. of pregnancies	*Abortion**		*Multiple pregnancy†*	
		No.	*%*	*No.*	*%*
Hepatitis B infection	27	5	19	5	23
No hepatitis B infection	21	6	29	4	27
TOTAL	48	11	23	9	24

Fisher's exact test: For percentage of abortion, $p = 0.5$; for percentage of multiple pregnancy, $p = 0.9$ (no significant differences).
*Ectopic pregnancy included.
†Percentage of multiple pregnancies among all pregnancies beyond first trimester.

There were 79 women with proved hepatitis B infection. Two of them already were in the convalescent phase when they became pregnant, and 24 women were pregnant and 55 were not during the infection. Seven of the pregnant women (29.2%) were slightly or severely icteric, and seven (29.2%) complained about arthralgia, whereas two (8.3%) were HBsAg positive for a long period of time. In the nonpregnant group, 24 (43.6%) were icteric, 35 (63.6%) complained about arthralgia, and two (3.6%) developed an HBV carrier state.

Prenatal diagnosis. Amniocentesis or biopsy of the chorion villi because of advanced maternal age (≥36 years) was performed in six women. With one exception, all were HBsAg negative at the time of prenatal diagnosis. The amniotic fluid of the woman who had amniocentesis performed while she was HBsAg and HBeAg positive was weakly HBsAg positive. Contamination with blood of the mother is the most likely explanation for this finding.

Incidents during pregnancy. As far as the course of pregnancy is concerned, three pregnancies need to be discussed.

Case 1. Routine ultrasonographic examination in the twenty-fourth week of pregnancy showed fetal ascites. An extended echoscopic evaluation showed that the child had not only ascites but a ventricular septum defect as well. There also was a polyhydramnion. The ascites spontaneously disappeared, and in the thirty-first week of pregnancy it could no longer be detected. By that time, however, multiple intraabdominal calcifications of unknown origin were seen. At 36.6 weeks' gestation a cesarean section was performed because of dysmaturity of the child. At birth no abnormalities other than dysmaturity (birth weight of the girl, 1785 gm) could be found. Prenatal closure of the ventricular septum defect was presumed. The mother was in the eighteenth week of pregnancy at the time of the first screening. At that time she was HBsAg positive, but she became HBsAg negative when she was 22 weeks pregnant.

Case 2. This was a triplet pregnancy that, with the exception of jaundice of the mother, was more or less uncomplicated until the twenty-ninth week. One of the children died in utero without obvious cause. The pregnancy continued as a twin pregnancy, and at the end of the thirty-sixth week two healthy children (a boy and a girl) were born. The mother was HBsAg positive at the time of conception and remained HBsAg positive until about 1.5 weeks before delivery.

Case 3. This was a singleton pregnancy that, with the exception of jaundice of the mother, was uncomplicated until the twenty-sixth week. By that time the woman was admitted to the hospital because premature rupture of the membranes was suspected. There also were premature uterine contractions and some vaginal bleeding. Bed rest and tocolytics managed to stabilize the condition. She was dismissed 3 weeks later. At the end of the thirty-seventh week, a healthy son was born. The mother was HBsAg positive at the time of conception and remained HBsAg positive until the twenty-second week.

Management at birth. The gynecologist who performed the prenatal controls was instructed about man-

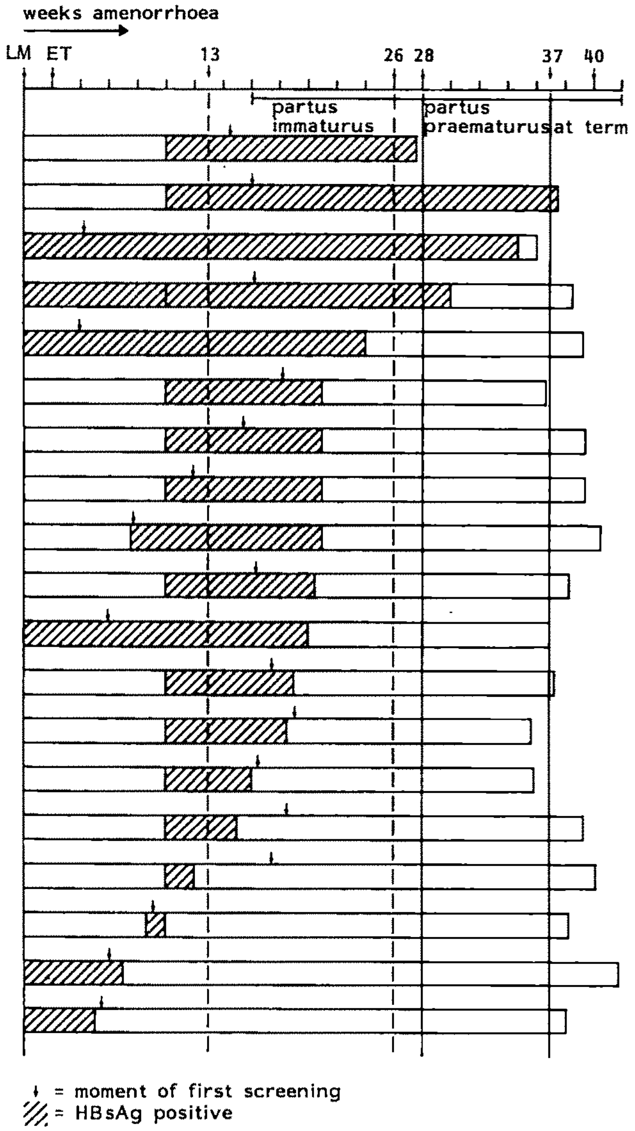

Fig. 2. Pregnancies with HBsAg-positive phase. Each *bar* represents a pregnancy, whereas HBsAg-positive phase is represented by *shaded* part. *Arrow,* Moment of first screening; *dotted lines,* end of first and second trimesters; *solid lines,* end period of immature and premature birth.

agement at birth. This consisted of (1) collecting 10 ml amniotic fluid, 10 ml of blood from the mother, and 20 ml of blood from the umbilical cord before delivery of the placenta, (2) administration of 250 IU HBIG to neonates of mothers who were HBsAg positive during the last 3 months of pregnancy or at the time of delivery, and (3) the scheme of follow-up by the pediatrician.

The babies were born between June 7 and Nov. 28, 1988. Duration of pregnancy varied between 27.6 and 41.6 weeks, and birth weight varied between 1075 and 4250 gm. Fig. 2 shows the period of pregnancy in which the women were HBsAg positive. Fourteen women were HBsAg negative at the time of conception but became contaminated during embryo transfer. The length of the incubation time of HBV infection is classically considered to range from 60 to 160 days.[9] Virus dose and inoculation route determine the incubation time.[10] After parenteral exposure, most cases of hepatitis appear during the first 50 to 60 days. Therefore the women were assumed to be HBsAg positive from the eighth week after embryo transfer. Five women were HBsAg positive at the time of conception. They also had been treated in period A but became pregnant after Jan. 21, 1988.

Two women were highly infectious (HBsAg and HBeAg positive) at the time of delivery. Two women were HBsAg negative at the time of delivery but HBsAg positive in the preceding 3 months. The eight children of these four women each received 250 IU HBIG within 2 hours of birth.[11, 12] Eleven women became HBsAg negative before the end of the second trimester and four before the end of the first. The hepatitis B infection had been cured at the time of conception in three women. Fifteen women had no sign of hepatitis B infection at all; six of them had been treated in period A and nine in period B.

One pregnancy (a triplet) ended in a partus immaturus (27.6 weeks). Fourteen children (one set of triplets, four sets of twins, and three singletons) were born before the end of the thirty-seventh week of pregnancy. The remaining 30 children (two sets of twins and 26 singletons) were born at term. In Table VI the number of immature, premature, and term births, and the number of children are shown in relation to hepatitis B infection of the mother during or before pregnancy. No significant differences between women with and without hepatitis B infection in percentage of immature, premature, and term births were detected.

Umbilical cord blood. Of the four children (immature triplet and term singleton) of the two women who were HBsAg and HBeAg positive at the time of delivery, the test results of the umbilical cord blood indicated the presence of HBsAg in three samples. Blood samples taken from these children some days post partum were HBsAg negative, so a perinatal hepatitis B infection in these children is very unlikely. Of the other 43 children, the test results of the umbilical cord blood were: HBsAg negative, anti-HBc negative, and anti-HBs positive in 20 cases; HBsAg negative, anti-HBc positive, and anti-HBs negative in two cases; and HBsAg negative, anti-HBc positive, and anti-HBs positive in 21 cases. The test results of umbilical cord blood and maternal blood were identical, indicating a passive diffusion of the anti-HBc and anti-HBs antibodies.

Perinatal death. Of the 49 children, 47 were born alive (25 girls, 22 boys). Of the immaturely born set of triplets (3 boys), one died 12 days post partum from septic candidiasis. Their mother was HBsAg and HBeAg positive at the time of delivery.

Two children were stillborn. One (a boy) was part of

Table V. Relation between amount of HBV introduced into uterine cavity, number of HBV infections and pregnancies resulting from these treatments, and number and percentage of abortions and multiple pregnancies

	Category 1		*Category 2*		*Category 3*	
	No.	*%*	*No.*	*%*	*No.*	*%*
Treatments* (embryo transfer)	36		44		6	
HBV infections	34	94	28	64	1	17
Pregnancies	8	22	10	23	2	33
Abortions	1	3	3	7	1	17
Multiple pregnancies	1	3	2	5	1	17

*One embryo transfer per woman, so number of embryo transfer is equal to number of women.

Table VI. Relationship between hepatitis B infection of mother and number and percentage of immature, premature, and term deliveries (births) and children

	Immature				*Premature*				*Term*			
	Births		*Children*		*Births*		*Children*		*Births*		*Children*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
No hepatitis B infection	0	0	0	0	4	27	8	40	11	73	12	60
Hepatitis B infection	1	5	3	11	4	18	6	22	17	77	18	67
TOTAL	1	3	3	6	8	22	14	30	28	76	30	64

χ^2 Test for percentage births, $p = 0.6$.

a triplet. He died in utero in the twenty-ninth week of pregnancy (see Incidents during pregnancy, case 2). The other one was part of a twin. The blood test of the mother indicated that she had a cured hepatitis B infection before she underwent IVF. At term, a healthy baby girl was born. The stillborn child had an extremely low birth weight, so it probably died in utero some weeks earlier.

Postnatal problems. Congenital malformation was not noticed in any of the children at birth. One boy, the youngest of the prematurely born triplet, was admitted to the hospital at the age of 6 weeks with a meningococcal meningitis. The pregnancy was the result of treatment in period A, contact of the preembryos with the HBV-containing culture medium was unlikely, and the mother had no signs of hepatitis B infection during pregnancy or after delivery. Another boy (born at term) was admitted to the hospital at the age of 2 weeks. He was suspected to have a sepsis, but a causative microorganism could not be found. The pregnancy was the result of treatment in period A, and the mother was 17.4 weeks pregnant when the hepatitis B epidemic was discovered. At the first screening her blood showed the serologic signs of a recently cured hepatitis B infection. The amount of anti-HBs indicated that she probably lost the HBsAg before the end of the first trimester. One girl (part of a prematurely born twin) was treated for cystitis. The pregnancy was the result of treatment in period A. At the time of first screening the mother was a little more than 19 weeks pregnant and had already entered the early recovery phase (HBsAg negative, anti-HBc positive, and anti-HBs negative). Another prematurely born girl (for details about the pregnancy see Incidents during pregnancy, case 1) was treated for otitis media. Three children (twins born at term and a girl born at term) had exanthema subitum. The mother of the twins was HBsAg positive at the time of conception but became HBsAg negative before the sixth week of pregnancy, while the mother of the other child already was in the recovery phase at the time of conception. Both pregnancies were the result of IVF treatment after Jan. 21, 1988. Two children (a boy and a girl both born at term) had cow's milk protein intolerance. One pregnancy was the result of treatment in period A. The mother was >18 weeks pregnant and already in the recovery phase at the time of the first screening. The other pregnancy was the result of treatment in period B. The mother received the full vaccination scheme.

Comment

Our main knowledge about the effect of the hepatitis B virus on the course of pregnancy and the unborn child stems from pregnancies in women who are HBV carriers or suffer from an acute hepatitis B infection during pregnancy.[13] When a woman is HBsAg positive at the time of conception, the oocyte can be exposed to the virus during ovulation and the preembryo while passing through the fallopian tube. It is presumed that the fetal organs become susceptible to HBV only in the third trimester. So, if the mother is HBsAg positive in the third trimester of pregnancy, there is a chance of prenatal infection. Placental leakages are thought to play an important role in HBV infections in utero.[14]

Children of women who are HBsAg positive at the time of birth, especially if they are also HBeAg positive, have a considerable chance of perinatal contamination.[15-17] The chances of becoming an HBV carrier are inversely correlated with age at the time of exposure to the virus.[18-20] To protect the newborn from neonatal HBV infection or from becoming an HBV carrier, the baby should be immunized immediately after birth.

A deficient clinical and laboratory protocol, together with insufficient instruction of the laboratory staff about the resistance of HBV to serum inactivation (30 minutes at 56° C), has led to the accidental infection described in this article.

The HBV contamination of IVF culture medium offered the opportunity to study the effect of exposure of a preembryo or fetus to the virus on pregnancy outcome but also pointed out the risk of using pooled serum. Contamination can be prevented by using only the patients' blood for preparing the culture medium. When use of donor serum is necessary, one should use the serum of a thoroughly screened (HBV and human immunodeficiency virus) female donor with proved fertility or fetal cord blood. A safe alternative is the use of human albumin solution or other serum substitutes. Much research is being done to find a substitute that is compatible with human serum.

The HBV exposure of the preembryo during IVF procedure did not result in any demonstrable damage, as may be concluded from the pregnancy rate per embryo transfer, the multiple pregnancy rate, and the rate of first-trimester abortion. The rates do not differ from those in periods without HBV contamination of the culture medium (Table II). Even if the preembryos were exposed to a relatively high dose of HBV, no negative effect on treatment and pregnancy outcome could be found, but the numbers are too low to allow a firm conclusion (Table V).

Likewise, the occurrence of a maternal hepatitis B infection during pregnancy did not seem to harm the embryo or developing fetus. In mothers with hepatitis B infection during pregnancy, the rate of multiple pregnancy, first-trimester abortion, immature birth, and premature birth did not differ significantly from those in mothers without hepatitis B infection during their pregnancy (Tables IV and VI).

In our observations there is no evidence for any harmful effect on fetus or newborn of either the exposure to HBV in the embryonic period or the occurrence of a maternal HBV infection during the first, second, or third trimester of pregnancy. In none of the total of 47 children born alive could a congenital HBV infection or a congenital malformation that could be related to HBV infection be demonstrated. In three children whose mothers were HBsAg and HBeAg positive at the time of delivery, the umbilical cord blood was HBsAg positive. In all three cases a blood sample taken some days post partum was HBsAg negative. It may be concluded that the umbilical cord blood of those children was contaminated with maternal HBsAg–positive blood. The intrauterine deaths of two children and the death of another child 12 days post partum were due to causes other than HBV infection. The same can be said of the children who became ill in the neonatal period. The clinical sepsis could not be explained by actual HBV infection, but some immunologic weakness resulting from HBV infection in the embryonic or fetal period cannot be excluded with certainty.

Theoretically, the absence of demonstrable or sustained presence of HBsAg in the neonatal blood does not prove that the HBV has not entered the fetal body early in pregnancy. Incorporation of (part of) the HBV deoxyribonucleic acid in the deoxyribonucleic acid of the fetal cells can be hypothesized to occur without a trace of HBsAg in the fetal blood or later in the neonatal blood. Tests to prove or deny this hypothesis, using molecular hybridization, are planned but not yet performed.

A long period of follow-up is needed, because problems relating to small defects may show themselves months to years after birth. Nevertheless, presently (11 to 15 months after birth) the observations on IVF offspring at risk of HBV influence are reassuring.

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Early detection of preeclampsia

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Amsterdam, The Netherlands, and Memphis, Tennessee

The signs and symptoms of preeclampsia are usually apparent at a relatively late stage in pregnancy (late second to early third trimester). However, the disorder results from abnormal interaction between fetal and maternal tissue much earlier in pregnancy, between 8 and 18 weeks' gestation. During the past two decades numerous clinical, biophysical, and biochemical tests have been proposed for the early detection of preeclampsia. Some of these tests are simple, whereas others are invasive; some have been studied extensively, while others are still under clinical investigation. A review of the literature indicates considerable disagreement regarding the sensitivity and predictive values of the various tests studied. The reported differences in the predictive values of these tests may be attributed to one or more of the following: populations studied, definition and prevalence of the disorder, techniques and methodology used in performing these tests, etc. As a result, there is disagreement regarding the ideal screening test to be used for identifying patients for clinical trials dealing with prevention of the disorder. (AM J OBSTET GYNECOL 1991;165:160-72.)

Key words: Preeclampsia, prediction, biophysical tests, biochemical tests

Preeclampsia is a common disorder of pregnancy and a major cause of maternal, fetal, and neonatal mortality and morbidity.[1-4] Thus its prevention would have a significant impact on maternal and perinatal outcome worldwide. Prevention not only requires knowledge of pathophysiologic mechanisms of disease, but also availability of methods of early detection, and means for intervention and correction of pathophysiologic changes.[5]

The signs and symptoms of preeclampsia become apparent at a relatively late stage in pregnancy, usually in the third trimester. However, the underlying cause of the pathophysiologic mechanisms that are thought to be responsible for the disease process appear to occur much earlier in pregnancy, between 8 and 18 weeks' gestation.[6] For that reason it seems logical to search for earlier indicators of this disorder,[7] and, indeed, a multitude of tests have been proposed as a means of predicting the later development of the disease (Table I). In reviewing the extensive literature on this subject one is faced with two major problems. One of these is the inconsistency in the terminology used to define or diagnose preeclampsia. Thus, for the purpose of this review, the term *preeclampsia* will be used to describe previously normotensive patients with persistent elevations in blood pressure appearing in the second half of pregnancy. The second major problem is the absence of internationally accepted criteria to judge the usefulness of a predictive test. Of course, the ideal predictive test should be simple and easy to perform early in pregnancy and be reproducible and noninvasive, with high sensitivity and a high positive predictive value. However, at this moment there is no one test that fulfills all the above criteria. In view of this, the actual value of an individual test used for early prediction of preeclampsia should be evaluated in the context of the clinical or research setting in which that particular test is used. For example, detailed longitudinal pathophysiologic studies, which may carry an appreciable burden for the study subjects, are possible only in rather small groups of high-risk patients selected by a test with a high predictive value. In this setting it is of lesser importance if the particular test is invasive, is time-consuming, or has only a moderate sensitivity. On the other hand, for a test to identify a large, high-risk group suitable for studying the effects of therapeutic intervention, the test needs to be inexpensive, easy to carry out, and noninvasive and express a high sensitivity. In these circumstances an average predictive value is acceptable.

In this review the various clinical, biochemical and biophysical tests that have been proposed to predict the development of this disease will be described, and their relative importance evaluated.

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Standard methods of antenatal care

Antenatal visits are conventionally arranged monthly between 20 and 28 weeks, biweekly up to 36 weeks, and weekly thereafter until delivery. These visits are dominated by a check of blood pressure and urine and by the determination of weight gain. The issue of whether the development of preeclampsia may be predicted on the basis of these standard variables has been addressed in several retrospective and some prospective studies.[8-19]

Blood pressure. A large prospective study in 808 nulliparous and 175 parous pregnant women was reported by Moutquin et al.[9] The investigators used an automatic oscillometric sphygmomanometer and started their blood pressure readings between 9 and 12 weeks' gestation. A total of 7800 blood pressure readings were obtained at 4-week intervals. Among 734 initially normotensive nulliparous women, preeclampsia (blood pressure $>135/85$ mm Hg, edema, or proteinuria after 20 weeks) developed in 46 (6.3%). The sensitivity for predicting preeclampsia early in pregnancy (9 to 20 weeks) on the basis of the occurrence of a diastolic blood pressure of ≥ 80 mm Hg was 32% to 46%; on the basis of ≥ 85 mm Hg it was 16% to 31%, with specificities of 84% to 91% and 93% to 97%, respectively. The data revealed that a majority of pregnant women who remained normotensive during pregnancy had a diastolic blood pressure of ≤ 75 mm Hg before 20 weeks' gestation. The sensitivity of using an 80 to 85 mm Hg diastolic blood pressure level in the first half of pregnancy as a test for the later occurrence of preeclampsia was poor. The predictive value of a positive test was on the order of 20% to 30%, but the predictive value of a negative test was approximately 95%.

Values of mean arterial pressure in the second trimester (18 to 26 weeks) have been used in predicting the development of preeclampsia later in pregnancy. Page and Christianson[10,11] presented data on the outcome of pregnancy on the basis of a prospective study of 14,833 single births. They found that when the average mean arterial pressure (MAP) in the midtrimester was ≥ 90 mm Hg there was a significant increase in the frequency of preeclampsia, fetal growth retardation, and stillbirths. On this basis, an average MAP value of 85 or 90 mm Hg in the second trimester has been used by several investigators to predict the later occurrence of preeclampsia.

The reports from which sufficient information could be obtained to calculate statistics for the test results are summarized in Table II.[9-19] Only one group of authors[14] stated that the results of second-trimester MAP measurements were withheld from the physicians and the patients. In addition, the definition of preeclampsia shows significant inconsistencies. It is usually not stated whether elevations of blood pressure occurring for the first time during labor or in the early postpartum period are included; in most studies proteinuric preeclampsia is not distinguished from transient hypertension. An exception is the carefully conducted study reported by Villar and Sibai.[17]

Table I. Tests proposed for early detection of pregnancy-induced hypertensive disease

1. Standard methods of antenatal care
    - Blood pressure increase
    - MAP in second trimester
    - Proteinuria by dipstick, microalbuminuria
    - Excessive weight gain, edema
2. Vasoconstriction tests
    - Cold pressor test
    - Flicker fusion test
    - Isometric exercise test
    - Peripheral venous tone test
    - Roll-over test
    - Infusion of catecholamines, vasopressin
    - Infusion of angiotensin II
3. Biochemical markers
    - Uric acid
    - Urinary calcium excretion
    - Urinary excretion of tubular enzymes
    - Urinary excretion of prostacyclin metabolites
    - Placental proteins
    - Various enzymes
    - Platelet angiotensin II receptors
    - Platelet calcium response to arginine vasopressin
    - Clearance of dehydroisoandrosterone sulfate
4. Hematologic markers
    - Plasma volume, hematocrit, hemoglobin
    - Plasma factor VIII–related antigen activity
    - Ratio of factor VIII–coagulant factor VIII
    - Antithrombin III levels
    - Thrombin–antithrombin III levels
    - Protein C
    - Plasminogen activator inhibitor 1
    - Fibronectin, ED1 + fibronectin
    - Platelet count, platelet volume
    - β-Thromboglobulin, platelet factor IV
5. Ultrasonographic evaluation
    - Doppler waveforms of uteroplacental circulation
    - Placental location

Villar and Sibai[17] studied 700 young, normotensive, primigravid women prospectively during pregnancy. In this study a second-trimester MAP of >90 mm Hg had a sensitivity of 8% and a positive predictive value of 23% (incidence of preeclampsia, 19.6%). The respective values for a threshold increase of >15 mm Hg in diastolic pressure were 39% and 32%. For a threshold increase of >30 mm Hg in systolic pressure, values were 22% and 33%. They concluded that neither a second-trimester MAP >90 mm Hg nor a threshold increase in systolic or diastolic pressure during the third trimester was significantly predictive of the development of preeclampsia.

The general impression is that a pregnant woman with a diastolic blood pressure of ≤ 70 mm Hg, or a mean arterial pressure of about ≤ 80 mm Hg in the

Table II. Summary of literature concerning predictive value of blood pressure in second trimester

Authors	*No. of cases*	*Parity*	*MAP ≥90 mm Hg*	*Preeclampsia*	*Sensitivity (%)*	*Specificity (%)*	*Predictive value*	
							Positive (%)	*Negative (%)*
Fallis and Langford[12]	113	Nulliparous	41	39	82	88	78	90
Page and Christianson[10, 11]	14,833	All parities	2014	399	43	87	8.6	98
Friedman and Neff[*, 13]	22,582	All parities	8807	888	64	62	6.4	98
Quaas et al.[14]	801	All parities	207	213	65	88	15	76
Phelan[15]	96	Nulliparous	7	15	20	95	43	87
Öney and Kaulhausen[16]	200	Nulliparous	85	21	90	62	20	98
Moutquin et al.[9]	808	Nulliparous	?	46	56-71	66-78	11-14	96-98
Chesley (unpublished data)	175	Nulliparous	9	2	0	97	0	99
Marya et al.[18]	200	All parities	37	28	32	84	24	88
Villar and Sibai[17]	700	Nulliparous	48	137	8	93	23	81

*The data of Friedman and Neff are for maximal second-trimester MAP.

second trimester runs a small risk of having preeclampsia later in pregnancy. The consequences of this rather obvious conclusion for antenatal care are minimal. The final conclusion is, as already stated by Chesley and Sibai,[19] that if an increased second-trimester MAP level predicts anything, it is transient hypertension rather than preeclampsia-eclampsia.

Proteinuria. In a recent investigation of 100 pregnant women with mixed parity attending an antenatal clinic for high-risk patients, Lopez-Espinoza et al.[20] studied microalbuminuria using a sensitive radioimmunoassay to determine whether an increasing loss of albumin would predict the occurrence of proteinuric preeclampsia. No evidence was found that gross proteinuria is preceded by a gradual increase in microalbuminuria. In another recent investigation of 88 normotensive pregnant women with mixed parity (11.4% incidence of preeclampsia), a urine albumin concentration of ≥11 μg/ml was reported to have a sensitivity of 50% and a specificity of 82%, a positive predictive value of 26%, and a negative predictive value of 93%.[21] The general conclusion is that there is little value in using precise techniques of detecting proteinuria to predict preeclampsia.[22-24]

Weight gain. In 1952 Hamlin[25] made the rather dogmatic statment that "if a young primipara with a low initial blood pressure increases her weight by more than 8 lbs. between the 20th and 30th week, she will proceed almost inexorably towards eclampsia." This led to renewed and vigorous manipulation of the diets of millions of pregnant women. Hamlin's statements were checked in a careful retrospective study in 1457 selected primigravid women in Aberdeen.[26] About two thirds of the normotensive women and three fourths of the women in whom preeclampsia developed gained more than 8 pounds between 20 and 30 weeks of pregnancy. The authors concluded that the relationship of weight gain between 20 and 30 weeks of pregnancy to preeclampsia is too indefinite to be of any practical value in predicting the development of preeclampsia. This conclusion was later supported by the results of another retrospective study, also from Abderdeen.[27] In conclusion, there appears to be such an important variability in physiologic weight gain in pregnancy and such an overlap between weight gains of normotensive and hypertensive pregnant populations that weight gain cannot be used to predict the development of preeclampsia.[28-30]

Biochemical and biophysical tests of vasoconstriction. A variety of tests have been devised to demonstrate the presence or absence of an abnormal vascular responsiveness before the clinical onset of the disorder (Table I). Most of these tests have only historic value and will be mentioned only briefly.

Isometric exercise test. Isometric hand-grip exercise is known to increase systemic arterial blood pressure, presumably resulting from increased systemic vascular resistance.[31] For this reason, Degani et al.[32] proposed that the diastolic blood pressure response to an isometric hand-grip exercise test might reflect vascular reactivity in pregnant women and thus might be used to detect vascular hyperreactivity and to predict preeclampsia.

One hundred healthy primigravid women were subjected to an isometric hand-grip exercise test between 28 and 32 weeks' gestation.[32] Each woman was placed in the left lateral position, and blood pressure was recorded at regular intervals until it remained stable. Blood pressure measurements were taken on the passive arm, and an increase in the diastolic pressure of ≥20 mm Hg was taken as a positive isometric pressor response. The results of the test were not made available to the physicians taking care of these women or making the clinical diagnosis. Eighty-four women had a negative test (preeclampsia developed in three of them) and 16 had a positive test (three remained normotensive). These results gave a sensitivity of 81% and

Table III. Validity of the roll-over test in reported studies

					Predictive value	
Authors	*No. of patients*	*Preeclampsia* incidence (%)*	*Sensitivity (%)*	*Specificity (%)*	*Positive (%)*	*Negative (%)*
Gant et al.,[34] 1974	38	45	88	95	93	91
Phelan et al.,[36] 1977	207	13	78	82	39	82
Marshall and Newman,[38] 1977	100	28	75	94	84	91
Gusdon et al.,[39] 1977	60	22	77	79	50	93
Andersen,[35] 1980	191	25	44	57	26	75
Verma et al.,[40] 1980	130	23	63	62	51	88
Kuntz,[42] 1980	65	38	60	68	54	73
Poland et al.,[45] 1980	139	32	42	62	34	69
Kassar et al.,[46] 1980	74	27	60	35	25	70
Thurnau et al.,[43] 1983	75	37	73	5	37	71
Öney and Kaulhausen,[52] 1983	188	14	63	75	30	92
Tunbridge,[48] 1983	100	19	10	90	20	81
Degani et al.,[32] 1985	100	9	67	57	13	95
Dekker et al.,[51] 1990†	90	13	25	92	33	89

*Main blood pressure criteria for diagnosis of preeclampsia: increase in diastolic blood pressure of ≥20 mm Hg and/or diastolic blood pressure ≥140/90 mm Hg.

†Pressor response 9 mm Hg defining a positive test. Pressor response 20 mm Hg = sensitivity of 0% and positive predictive value of 0%.

specificity of 96%. The positive predictive value was 81%, and the negative predictive value was 93%, with an incidence of preeclampsia of 16%.

Marya et al.[18] also reported on the predictive value of the isometric exercise test. They studied 200 women with mixed parity. Sensitivity of the test was reported to be 54%, specificity 98%, positive preditive value 79%, and negative predictive value 93% (incidence of preeclampsia, 14%). However, the authors did not report the definitions that were used for diagnosing preeclampsia. In conclusion, judgment on the isometric exercise test as a predictor of preeclampsia awaits the results of further carefully conducted prospective studies to support or refute its validity.

Roll-over test. The roll-over or supine pressor test was first described by Gant et al.[33] The test is performed between 28 and 32 weeks of pregnancy with the woman lying on her left side until the diastolic blood pressure, measured in the uppermost right arm, is stable. The woman is then turned on her back, and diastolic blood pressure is again recorded. A rise in diastolic blood pressure of ≥20 mm Hg defines a positive test. The first study comprised 50 randomly selected, healthy primigravid women. Pregnancy outcome was given for only 38 of the 50 women; apparently 12 had not completed their pregnancies when the report was published. Of 16 women with a positive roll-over test, pregnancy-induced hypertension developed in 15; of 22 women with a negative roll-over test, 20 remained normotensive. The prevalence of preeclampsia in this unselected sample of primigravid women was 44.7%. Since this original report, many reports have been published on this subject, which included >1500 patients.[7, 18, 20, 34-51] Results of pertinent studies are summarized in Table III. The sensitivity and specificity values of about 90% that can be calculated from the results of Gant et al.[34] were not confirmed in the majority of the other studies. Various factors may explain the marked disparity in results of a relatively simple test as the roll-over test. Although all investigators define an increase in diastolic blood pressure of ≥20 mm Hg as a positive test, there was considerable variation among investigators in the method of the test[15] and the measurement of blood pressure (using Korotkoff phase 4, phase 5, or Doppler-shift signals). In addition, there were the usual inconsistencies in the definition of preeclampsia. Moreover, most authors, with only a few exceptions, failed to identify the arm used to record blood pressure. This is of great importance, since turning the patient from the left side on to her back with blood pressure recorded from the right (superior) arm will result in a predictable increase in diastolic pressure of approximately 10 to 12 mm Hg because of the increase in hydrostatic pressure relative to the level of the heart.[53] Furthermore, it is uncertain in most reports whether tests were assessed in a double-blind fashion. Several of the publications state that the results of the tests were not revealed to the physicians in charge or to the patients themselves, but it is not mentioned specifically whether the diagnosis of preeclampsia was made without knowledge of the test results. In summary, the results of the roll-over test are highly variable among different investigators, and there is poor reproducibility in the same patient. Studies in the literature report sensitivities varying between 0% and 93%, specificities between 54% and 91%, and false-positive results up to 90%. The final conclusion must be that, although the roll-over test has gained some popularity

Table IV. Validity of angiotensin sensitivity test in reported studies

Authors	EPD	No. of patients	Duration of gestation (wk)	Preeclampsia incidence (%)	Sensitivity (%)	Specificity (%)	Predictive value: Positive (%)	Predictive value: Negative (%)
Gant et al.,[33] 1973	<8	192	28	38	?	?	86	94
Morris et al.,[59] 1978*	<8	26	29-32	12	33	39	7	82
	<10				67	30	11	87
Orozco et al.,[64] 1979*	<8	33	28-32	27	89	79	62	95
Öney and Kaulhausen,[52] 1982	<08	231	28-32	15	76	83	45	95
Nakamura et al.,[65] 1986	<8	48	30	21	20	97	75	82
	<10			80	82		53	94
	<12				100	74	50	100
Dekker et al.,[51] 1990	≤8	90	28	13	75	99	90	9
	≤10	90	28	13	92	86	50	99

EPD, Effective pressor dose below which test was considered positive.
*At least one positive test in serial testing.

because of its simplicity, it is of little use clinically as a predictive test. However, it may be useful to screen patients for study purposes.

Infusion of epinephrine and norepinephrine. The pressor effects of epinephrine infused in physiologic[54] and pharmacologic[55-57] doses appear to be more pronounced in women with mild preeclampsia than in normotensive pregnant women. Raab et al.[54] investigated the pressor effects of two doses of epinephrine in 163 normotensive pregnant women between 28 and 34 weeks' gestation. They noted an increase in systolic blood pressure that was higher in women who became hypertensive later in pregnancy than in women who remained normotensive. However, there was extreme variability in blood pressure responses and considerable overlap between responses of normal and abnormal groups. Also, the pressor response to norepinephrine appears to be enhanced in hypertensive pregnant patients as compared with normotensive pregnant women.[55-57]

Infusion of angiotensin II. In 1961, Abdul Karim and Assali[58] reported that intravenous infusion of angiotensin II (angiotonin) caused a smaller rise in blood pressure in pregnant women than in nonpregnant women. The relative refractoriness to the pressor effects of angiotensin II in normal pregnancy was later confirmed in several studies.[59-61] In addition, it was found that this refractoriness is lost to a marked extent in women with preeclampsia.[61, 62] Gant et al.[33] used the determination of the sensitivity to the pressor effects of infused angiotensin II to predict the development of preeclampsia. The study included 192 randomly selected young (13 to 17 years old) primigravid and 10 nonpregnant gynecologic patients. The authors first determined the amount of angiotensin II infused per kilogram of body weight per minute needed to raise the diastolic blood pressure by 20 mm Hg. The average so-called effective pressor dose was found to be about 8 ng/kg/min in nonpregnant individuals and in early (7 to 10 weeks) pregnancy. The required dose needed was approximately twofold (15 ng/kg/min) by 28 weeks and fell gradually thereafter to a level of 11 to 12 ng/kg/min at term. Up to about 20 weeks' gestation, all pregnant women showed this refractoriness to the pressor effects of angiotensin II that was thought to be a physiologic feature of pregnancy. However, in women in whom preeclampsia ultimately developed, the refractoriness gradually decreased and between 28 and 32 weeks the effective pressor dose had returned to nonpregnant values. Of the total group of 192 pregnant women, 153 underwent at least one angiotensin II infusion test between the twenty-eighth and thirty-second week. Of these, 103 remained normotensive and 50 subsequently developed preeclampsia in the third trimester. Of the 103 women who remained normotensive, 13 had an effective pressor dose of <8 ng/kg/min (positive test); of the 50 women who became hypertensive, 45 (90%) had at least one positive test.

The angiotensin sensitivity test is a rather complicated and time-consuming procedure, which may be the reason that its validity in predicting preeclampsia has been subsequently assessed in very few clinical investigations. There is one other study from the United States,[63] one from Mexico,[64] two from Europe,[51, 52] and one from Japan.[65] The results of these studies are summarized in Table IV. The numbers are small, and the definitions of a positive test and of preeclampsia are different. Thus it is very difficult to compare these studies.

In conclusion, the angiotensin sensitivity test is too complicated and time consuming to be used as a clinical screening procedure. It may, however, be useful at 28 weeks' gestation for research purposes to distinguish between patients with a high risk of approximately 50% and those with a low risk of 1% to 5% of preeclampsia, assuming an incidence of 10% to 15%.[51, 66]

Biochemical and hematologic markers. Concentrations of a large number of constituents of blood and urine, including hormones, have been shown to change in preeclampsia. Some of these changes have been proposed to be of value in the prediction of early diagnosis of the disorder and some of them will be discussed in the following paragraphs.

Broughton Pipkin et al. (Broughton Pipkin F, Baker PN, Symonds EM. Platelet angiotensin II receptors in predicting preeclampsia. Personal communication) recently studied platelet angiotensin II receptors in normotensive and hypertensive pregnancies. Platelets share various characteristics with vascular smooth muscle cells and are increasingly being used as readily accessible models for smooth muscle cells. This study showed that a physiologic fall in platelet angiotensin II binding occurs in early pregnancy, which is parallel to that of the vascular response to angiotensin II. It was found that platelet angiotensin II receptors are increased in preeclampsia, probably as an expression of the altered angiotensin II levels; this increase was shown to precede the increase in blood pressure. The Nottingham group reported a positive predictive value of 77.8%, a negative predictive value of 82.9%, a sensitivity of 87.5%, and a specificity of 86.7% for nulliparous women ($n = 23$) in predicting preeclampsia between 26 and 32 weeks' gestation. Also, recently an increase in the sensitivity of platelet calcium to arginine vasopressin was proposed to be useful as an early predictor of subsequent preeclampsia.[67] It was shown that the sensitivity of platelet intracellular calcium levels to arginine vasopressin in patients with subsequent preeclampsia is already increased at end of the first trimester, as compared with normotensive pregnancies.

Both these studies on platelet pathophysiologic characteristics underline the concept of preeclampsia as a trophoblast-dependent process that is mediated by a disturbed platelet-endothelium interaction. Although the potential value of platelet angiotensin II receptors and the increase in the sensitivity of platelet calcium to arginine vasopressin have been assessed only in small numbers of patients, these two methods hold great promise for studying cardiovascular pathophysiologic parameters in normotensive and hypertensive pregnancies and for the development of a predictive test.

Plasma volume, hemoglobin concentration, and hematocrit. It has been reported by many authors that the average plasma volume is less in patients with preeclampsia.[68] There is evidence that plasma volume contraction may precede the blood pressure rise in preeclampsia at least in some of the cases[69-71]; however, the numbers of patients studied are small, and there is considerable overlap between values of plasma volume in normal and abnormal groups. In addition, dye-dilution techniques for estimating plasma volume are subject to serious criticism,[72] and they are too complicated to be used as a screening procedure in standard antenatal care. The contraction of plasma volume that is often observed in preeclampsia may be revealed by an elevation of or a rise in hemoglobin concentration or hematocrit.[73] In addition, many obstetricians use serial measurements of the hematocrit to monitor the clinical course of preeclampsia. In a large retrospective study comprising >54,000 singleton pregnancies, Murphy et al.[74] showed a highly significant correlation in nulliparous and parous women between the frequency of occurrence of preeclampsia and the hemoglobin concentration at first visit before 24 weeks. In primigravid women, the frequency of subsequent preeclampsia ranged from 7% at hemoglobin values <10.5 gm/dl to 42% at hemoglobin concentrations >14.5 gm/dl.

Uric acid. In 1925, Stander et al.[75] reported increased serum uric acid levels in women with preeclampsia. Many later investigators confirmed this finding and demonstrated a positive correlation between the magnitude of serum uric acid levels, clinical severity of preeclampsia, and perinatal outcome.[76-79] On the basis of the results of a prospective (but not blinded study) in 332 hypertensive pregnant patients with mixed parity, Redman et al.[78] concluded that plasma uric acid levels constitute a better indicator than blood pressure as a prognosis for fetal outcome. However, other studies did not confirm these findings.[80]

The results of many studies[81-83] but not of others[84] in normotensive pregnant women suggest that serum uric acid levels may begin to rise before the appearance of hypertension and proteinuria. However, patients studied included nulliparous and parous women, some of them with renal disease or treated chronic hypertension, and the presentation of the data does not allow a reliable evaluation of the results. Moreover, the predictive value of uric acid determination was not assessed in a double-blind fashion in any of these studies.

In conclusion, as for other signs of preeclampsia, hyperuricemia is nonspecific. As yet there is no evidence that determination of uric acid levels can be used as a screening test to predict the later development of the disease. In patients with established preeclampsia, serum uric acid levels may reflect severity of the disease process. For that reason, uric acid levels can be used in the clinical management of these patients to monitor the course of the disease.

Urinary calcium excretion. Taufield et al.[85] reported that established preeclampsia is associated with hypocalciuria. The authors investigated a group of 40 pregnant women, 20 nulliparous and 20 multiparous, in the third trimester of pregnancy; 10 were normotensive, five had transient hypertension, six had chronic hypertension, six had chronic hypertension with superimposed preeclampsia, and 12 had preeclampsia. Urinary calcium excretion was not different between normotensive women and patients with transient or chronic hypertension, but it was significantly lower (13% to 25% of normal pregnant values) in preeclamptic patients. In these patients the fractional excretion of calcium was also reduced, suggesting increased tubular reabsorption.

In a recent investigation in 88 pregnant women with mixed parity (11.4% incidence of preeclampsia), Rodriguez et al.[21] assessed the value of the urinary calcium/creatinine ratio between 24 and 34 weeks' gestation. A calcium creatinine ratio of ≤ 0.04 was reported to have a sensitivity of 70%, a specificity of 95%, a positive predictive value of 64%, and a negative predictive value of 96%. In contrast, Roelofsen et al.[86] reported no difference in urinary excretion rates of calcium between normotensive and hypertensive pregnant women. The findings of these authors may be caused by the fact that they studied a very heterogeneous population. It is important to note that the results of Taufield et al.[85] were recently confirmed by Huikeshoeven and Zuijderhoudt.[87] Further prospective studies are needed to support or refute these findings and to determine whether a decrease in urinary calcium excretion could be used as a predictor of the disease.

Urinary excretion of prostacyclin metabolites. Evidence is accumulating that a relative deficiency of vascular prostacyclin plays an important role in the development of preeclampsia.[5] Fitzgerald et al.[88] reported that a reduction in the urinary excretion of 2,3-dinor-6-keto-prostaglandin F_1, a major metabolite of prostacyclin, precedes the development of clinical disease. Urine was collected at 11 to 20 weeks' gestation, 20 to 29 weeks' gestation, and between 28 weeks' gestation and term in 67 pregnant women considered to be at risk for preeclampsia. Patients were retrospectively allocated to one of four predefined groups (preeclampsia, chronic hypertension, hypertension in labor, or normotensive) by observers who were blinded as to the results of the biochemical analysis. Excretion of 2,3-dinor-6-keto-prostaglandin F_1 was increased throughout pregnancy in all pregnant women, as compared with that in normotensive nonpregnant subjects. However, 2,3-dinor-6-keto-prostaglandin F_1 excretion was significantly reduced in 12 women (18%) in whom preeclampsia developed later in pregnancy, as compared with that in pregnant women who remained normotensive or who became hypertensive during labor. In this study they found no difference between normotensive subjects and patients with pregnancy-induced hypertension in their pressor response to angiotensin II, despite a difference in prostacyclin biosynthesis. The authors stated that excretion of <400 pg 2,3-dinor-6-keto-prostaglandin F_1 per milligram creatinine at 20 to 28 weeks' gestation was associated with a 65% risk of preeclampsia, but they did not provide data to calculate sensitivity and specificity. A study of a larger population is needed to assess the predictive power of determination of urinary 2,3-dinor-6-keto-prostaglandin F_1 excretion.

Enzymes and hormones. Concentrations of various enzymes in serum or plasma have been studied in patients with preeclampsia, mainly to assess placental function.[3] After a brief period of clinical enthusiasm, none of these enzymes has been shown to be of use as an early indicator of hypertensive disease in pregnancy or as a biochemical measure of its severity.

As an extension of earlier studies,[82, 89] other investigators[90, 91] have adduced evidence that serum levels of deoxycytidylate deaminase are increased if preeclampsia develops but not in chronic hypertension. These investigators performed at least two determinations of deoxycytidylate deaminase, one at first visit and one in the third trimester, in sera of 2460 pregnant women, the parity of whom was not reported. An abnormally high value was defined as a value ≥ 2 SD above the mean of control values obtained in apparently normal pregnancies. A total of 133 women (5.4%) had pregnancy-induced hypertension or preeclampsia. All of 73 patients with moderate to severe preeclampsia and 52 of 60 women with mild disease had abnormally high levels. High levels were also observed in nine patients with a hepatic disorder. Only 53 other patients had high deoxycytidylate deaminase levels (false positive). It should be emphasized that the majority of the patients already had clinical signs of preeclampsia at the time of determination of deoxycytidylate deaminase values. No abnormally elevated levels were found before 24 weeks' amenorrhea and very few ($n = 4$) before 28 weeks, which makes it unlikely that this test can be used to predict the subsequent development of preeclampsia.

Wood and Durham[92] measured plasma cystyl aminopeptidase (oxytocinase) from 28 weeks' gestation until delivery in 92 primigravid women and reported significantly higher levels of the enzyme at 30 and 32 weeks in women in whom preeclampsia developed later. Further studies of plasma cystyl aminopeptidase in a larger number of patients are required to evaluate the possible predictive value of this plasma enzyme.

It has been claimed that the levels of the placental protein hormone termed *pregnancy-associated plasma protein A* are raised toward the end of the second trimester in women who are likely to have preeclampsia.[92-94] In a more recent study, it was found that measurement of the placental proteins pregnancy-associated plasma protein A, human placental lactogen, and SP1 (Schwangerschaft protein) is of no use in predicting the later development of preeclampsia.[95] Some studies suggested that β-human chorionic gonadotropin determination may have value for early detection of preeclampsia. However, because levels in both normal and preeclamptic pregnancies show a larger scatter and overlap, the practical usefulness of β-human chorionic gonadotropin determination as a predictive test seems limited.[96, 97]

The rate at which the placenta clears maternal blood of isotope-labeled dehydroisoandrosterone sulfate for estradiol synthesis has been postulated to reflect uteroplacental perfusion.[98] A longitudinal study of the metabolic clearance rate of this substance in women who developed preeclampsia later in pregnancy showed that during the first half to two thirds of pregnancy placental perfusion was similar to or even above that in women who remained normotensive. The metabolic clearance rate began to fall 2 to 4 weeks before hypertension was detected, by which time it was only 35% to 50% of normal.[99] These results have not been confirmed in other studies. In addition, the late occurrence of a fall in metabolic clearance rate of dehydroisoandrosterone sulfate and the important interindividual variation make it unlikely that this test may be used for prediction purposes.

Coagulation factors and platelets. Evidence for the occurrence of abnormal coagulation processes and platelet activation was originally based on the finding, as early as 1893, of fibrin deposits and thrombi in vessels of various organs of women who died of eclampsia. In later years, all factors of the extrinsic and intrinsic coagulation system have been extensively studied in women with preeclampsia, either directly or indirectly by means of various clotting tests. A complicated coagulation index has been proposed to predict the clinical progress of preeclampsia.[100] These studies have recently been reviewed and analyzed in detail.[101] The results are generally equivocal and do not suggest marked consumption of coagulation factors in most patients with preeclampsia. The most frequently occurring hemostatic abnormalities in patients with preeclampsia are a rise in the factor VIII–related antigen level and a reduced antithrombin III concentration.

Thrombin-induced coagulation, vascular endothelial damage, and platelet aggregation increase the circulating levels of factor VIII–related antigen (von Willebrand factor) and the ratio of that antigen to coagulant factor VIII. Some authors have reported an early rise of these factors in preeclampsia[102] whereas others have demonstrated a positive correlation between plasma factor VIII–related antigen activity or the level of the ratio of factor VIII–related antigen and coagulant factor VIII and the clinical severity of the disorder, growth retardation, and perinatal mortality.[103-108] However, there appears to be a particularly good correlation between plasma factor VIII–related antigen activity and the occurrence of fetal growth retardation. It can be concluded from these studies that finding this antigen or the factor VIII–related antigen-to-coagulant factor C ratio elevated constitutes a useful and quite early indicator of both the severity and the degree of placental insufficiency.

Decreased levels of antithrombin III have been demonstrated in a majority of patients with preeclampsia but not in pregnant women with chronic hypertension.[109, 110] However, early antithrombin III determinations had no value in predicting the development of preeclampsia later in pregnancy.[111] In addition, Dekker[5] found no difference in antithrombin III activity between angiotensin II–sensitive and angiotensin II–insensitive pregnant women at 28 weeks' gestation.

de Boer et al.[112] observed thrombin/antithrombin III levels more than twice as high in preeclampsia as those in gestational age–matched control subjects. Also, protein C levels were reduced substantially compared with normal pregnancy. However, there are no data on thrombin/antithrombin III and protein C levels before the onset of preeclampsia. Thus further prospective longitudinal studies are needed to evaluate their possible predictive value.

Recently attention has been focused on plasma levels of fibronectin, a glycoprotein involved in coagulation, platelet function, tissue repair, and the vascular endothelial basement membrane. Fibronectin levels were found to be markedly elevated in preeclamptic patients[113-116] and correlated with low antithrombin III levels and with the degree of proteinuria.

Ballegeer et al.[111] measured fibronectin and plasminogen activator inhibitor-1 levels in 120 normotensive primigravid women during the first, second, and third trimesters of pregnancy. Thirty-two women (26.6%) had preeclampsia. Increased levels of fibronectin at 25 to 36 weeks of pregnancy (greater than or equal to mean + 2 SD of the healthy women, or >140%) were found in 31 of the 32 women with hypertension (sensitivity 96%, specificity 94%). Fibronectin levels increased 3.6 ± 1.9 weeks earlier than the onset of hypertension or proteinuria. Increased levels of plasminogen activator inhibitor-1 at 25 to 32 weeks (≥280 ng/ml) were found in 16 of the 32 women (sensitivity 50%, specificity 95%). The authors concluded that increased fibronectin levels are the best predictor of preeclampsia and that its level in plasma increases several weeks before the development of hypertension.

Lockwood and Peters[117] reported that increased plasma levels of ED1+ (extra type III domain) cellular fibronectin, almost exclusively localized to endothelium, may precede the clinical signs of preeclampsia. They found that ED1+ fibronectin levels were significantly higher in the preeclampsia group in the first and second trimesters, before clinical evidence of the disorder. The data are very promising but do not allow an assessment of the predictive value of this new biochemical marker.

Many reports in the literature indicate that in patients with preeclampsia the platelet count is lower than in uncomplicated pregnancies[118-120] and that thrombocytopenia may occur as an early[120] but also as a relatively late feature of the disorder. In addition, thrombocytopenia is not a feature in pregnant patients with chronic hypertension without superimposed preeclampsia.[121] However, platelet behavior in women with preeclampsia is extremely variable, and reduced platelet counts are not found in all cases, not even in eclampsia.[80] Results of various large studies suggest that approximately 20% of patients with preeclampsia have thrombocytopenia, varying between 7% in mild preeclampsia and 50% in severe preeclampsia-eclampsia.[119] Thrombocytopenia is usually mild; in some cases it may be accompanied by hemolysis and elevated liver enzymes, a typical constellation of abnormalities that has been named the HELLP syndrome.[121] Episodes of platelet activation and consumption are also reflected in increased plasma levels of β-thromboglobulin[122-124] and platelet factor IV[125] in some patients with preeclampsia but not with chronic hypertension. However, the results of these studies indicate that platelet behavior in preeclampsia is extremely variable, between patients and within one patient.

In conclusion, determination of factor VIII–related antigen activity or its ratio to coagulant factor VIII, plasma antithrombin III concentrations, and platelet counts may be of value in the management of preeclampsia. However, there is no convincing evidence that these determinations are of any value for prediction or early diagnosis of the disease. Presently, the measurement of total fibronectin or ED1 + fibronectin holds the greatest promise as a predictive test for the development of preeclampsia.

Doppler ultrasonography methods. The development of continuous wave and pulsed range-gaged Doppler ultrasonographic systems has made it possible to study the perfusion of deep-lying vessels such as the arcuate arteries and other branches of the uterine vasculature.[126-129] Because of the as yet inevitable errors in the measurement of volume flow, most emphasis has been put on the quantitative examination of flow velocity waveforms. The velocity waveforms in the branches of the uterine arteries in normal pregnancy show an increase in end-diastolic flow velocity between 14 and 20 weeks; there appears to be no further change in this pattern of low pulsatility and high diastolic velocity until term.[128-130]

In their first study, Campbell et al.[127] observed arcuate artery waveform patterns with reduced end-diastolic velocities suggestive of an elevated uteroplacental vascular resistance in 14 of 31 patients with complicated pregnancies. Of these 14 patients, 10 had preeclampsia and eight were eventually delivered of a small-for-gestational-age infant. Of the 17 patients with normal arcuate artery flow velocity waveforms, only four had preeclampsia, and six of the fetuses were growth retarded. The results of this study were later supported by those of a larger, nonblinded study by Trudinger et al.[129] On the contrary, Hanretty and Whittle[131] found no overall difference in uteroplacental waveforms between women with normal pregnancies and those with established preeclampsia.

The first prospective study in which the predictive value was determined of the occurrence of abnormal arcuate artery waveforms in the second trimester has been reported by Campbell et al.[127] A group of 149 unselected pregnant women was investigated; 17 were delivered elsewhere and there were incomplete records for six cases; of the remaining 126 women, 41 were nulliparous. Arcuate artery waveforms were determined between 16 and 18 weeks' gestation, and the clinicians who took care of the patients were not informed about the results. Of the 76 women with normal waveforms, preeclampsia developed in five and five other pregnancies were complicated by isolated fetal growth retardation. Of the 50 women with abnormal waveforms, preeclampsia developed in 10 and 10 pregnancies were complicated by fetal growth retardation alone. The sensitivity of determination of arcuate artery waveforms at 16 to 18 weeks' gestation as a test to predict preeclampsia or fetal growth retardation was 68%; specificity was 69%. The predictive value of a positive test was 42% and of a negative test 87%. The authors did not present an explanation of the remarkable finding of 40% abnormal waveforms in an unselected sample of pregnant patients or of the high incidence (24%) of complicated pregnancies. Similar results were reported by Arduini et al.[132]; they studied 60 high-risk pregnancies at 18 to 20 weeks' gestation (incidence of preeclampsia, 36.6%). The validity of uteroplacental waveforms analysis was as follows: sensitivity, 63.6%; specificity, 84.2%, positive predictive value, 70%, negative predictive value, 80%.

In a study from the Fetal Welfare Laboratory at St. George's Hospital, London, the results of Doppler ultrasonography as a screening test for any degree of hypertension were somewhat disappointing, with a sensitivity of only 25% at 24 weeks but with a sensitivity of nearly 100% for severe preeclampsia or fetal growth retardation.[133, 134]

A predictive value of about 50% reported in the studies of Campbell et al.[128] and Steel et al.[134] are not approached by several recent studies. Although Newnham et al.[135] reported a significant association between uteroplacental systolic/diastolic ratios at 24 weeks' gestation and subsequent fetal hypoxia (sensitivity, 24%; specificity 93.9%), they could not find a relationship between elevated systolic/diastolic ratios and preeclampsia. In addition, Jacobson et al.[136] studied the value of Doppler assessment of the uteroplacental circulation in predicting preeclampsia or fetal growth retardation in 136 high-risk women. In this study a low fetal abdominal circumference at 20 to 24 weeks or an increasing maternal plasma uric acid concentration at 24 weeks was as predictive as an elevated resistance index. The authors concluded that although elevated resistance indices occur more commonly in women with fetal growth retardation or preeclampsia, the correlation is not close enough to be clinically useful as a screening test.

Measurement of arcuate artery blood flow velocities between 16 and 24 weeks' gestation may diagnose impaired trophoblast invasion of the spiral arteries and may therefore have great potential in predicting pregnancies destined to become complicated by preeclampsia or fetal growth retardation. In addition, this method may be useful to monitor the course of the hypertensive disorder and the effects of treatment. Probably Doppler ultrasonography of the uteroplacental circulation is useful only as a predictive test for the later development of severe preeclampsia or fetal growth retardation,[132-134] but this may be clinically important because most hypertension in pregnancy probably represents an enhanced physiologic adaptation to pregnancy.

Even more simple ultrasonographic techniques may be of use in identifying women with a high risk of hypertension in pregnancy. Koffinas et al.[137] showed that patients with unilateral placentas have a 2.8-fold greater incidence of preeclampsia than women with central placentas. These results are supported by Schulman et al.[138] In this study it was reported that women with preeclampsia have a dominant unilateral uteroplacental blood flow.

Comment

More than 100 clinical, biophysical, and biochemical tests have been recommended for predicting the development of preeclampsia. The findings of numerous studies have been inconsistent and contradictory, owing to the heterogeneity of the populations studied and variations in the definitions used for hypertensive disorders during pregnancy and in the methods used to express results. Future studies should adhere to strict criteria to diagnose and classify hypertension during pregnancy. In addition, only healthy, nulliparous pregnant women should be included.

From this review it is obvious that currently there is no ideal predictive test that fulfills all desired criteria. The value of the roll-over test remains controversial at the least. The angiotensin sensitivity test is useless in clinical practice, but it may be considered useful for research purposes because it can be done in early pregnancy and has a high predictive value. An increased second-trimester MAP only predicts transient hypertension instead of the real disease, preeclampsia-eclampsia.

The ultimate goal in the management of preeclampsia is to be able to detect the disease in the the early stages and have available a therapy that either cures it or at least ameliorates its progression in an attempt to achieve fetal maturity. Concerning the development of an easy, reliable predictive test for the development of severe preeclampsia, the focus of attention is on total or ED 1+ fibronectin and Doppler-flow studies of the uteroplacental circulation. Doppler-flow studies of the uteroplacental circulation express a low to average predictive value; however, they are easy to carry out, inexpensive, and noninvasive. In addition, Doppler-flow studies can be done in early pregnancy and show a high sensitivity for severe preeclampsia with associated perinatal or maternal morbidity or mortality. Therefore Doppler-flow studies of the uteroplacental circulation are very suitable for therapeutic intervention to reduce the incidence of preeclampsia and its complications. The recent study on ED 1+ fibronectin demonstrating an increase in ED 1+ fibronectin in preeclampsia already occurring in the first and second trimesters before any clinical evidence of disorder is extremely promising. Also, the recent use of platelet angiotensin II receptors as an alternative, inexpensive and practical angiotensin sensitivity test and the increased platelet calcium response to arginine vasopressin may offer great promise in the future.

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Effects of maternally administered epidermal growth factor on placental permeability

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Epidermal growth factor is a well-studied modulator of epithelial membrane structure and function. Mammalian placentas are a rich source of epidermal growth factor receptors, but the role of epidermal growth factor in placental pathophysiologic conditions is unclear. To determine whether epidermal growth factor could affect mechanisms of placental transfer, we used an in situ rat placental perfusion model. Fourteen Sprague-Dawley rats that were 20 days pregnant were randomized to epidermal growth factor or placebo during placental transport experiments. We chose ethylenediaminetetraacetate tagged with chromium 51 as a marker of placental permeability. Epidermal growth factor treatment led to a dramatic increase of maternofetal clearances of chromium 51–ethylenediaminetetraacetate. We conclude that maternally administered epidermal growth factor has a potent action on placental permeability. (AM J OBSTET GYNECOL 1991;165:173-6.)

Key words: Epidermal growth factor, placenta, placental transport

Because of the importance of the placenta in maintaining mammalian pregnancy, there is increasing interest in biochemical factors that regulate placental function and allow the placenta to act as a critical interface between mother and fetus. Recently it has been recognized that the placenta is a rich source of growth factors and growth factor receptors,[1] as well as protooncogenes.[2] These findings have led to the suggestion that these molecular regulators play an important role in the control of placental or fetal growth. One of the best-studied growth factors in this regard is epidermal growth factor. Although the human placenta is one of the richest known sources of epidermal growth factor receptor,[3] the roles of epidermal growth factor and that of the structurally homologous protein transforming growth factor-α remain unclear.

In the placenta of normal, near-term fetal rats,[4, 5] there is a developmental increase in epidermal growth factor–binding sites; this is also the case for the human placenta during the last half of gestation.[6, 7] Placental membranes of intrauterine growth–restricted rats have increased binding of epidermal growth factor,[5] whereas placentas from streptozocin-treated diabetic rats have decreased the binding of epidermal growth factor.[4]

Since epidermal growth factor has been demonstrated to influence ionic signal transduction (sodium-hydrogen exchange)[8] and to increase transport of α-aminoisobutyrate[9] and deoxyglucose[10] in isolated cell culture systems, we undertook a study of the effect of maternally administered epidermal growth factor on placental transport. As an index of placental permeability, we selected chromium 51–ethylenediaminetetraacetate (EDTA), a known diffusion marker, believed to travel across the placenta by means of "small pores,"[11] because of its small molecular size. We used an in situ system of placental perfusion in the rat[12] to test the null hypothesis that maternally administered epidermal growth factor would not affect placental transport of ^{51}Cr-EDTA.

Material and methods

All chemicals were reagent grade, purchased from Sigma Chemical Co. (St. Louis). Radioisotopes were purchased from New England Nuclear (Boston). Receptor-grade epidermal growth factor was purchased from Bioproducts for Science (Indianapolis). Epidermal growth factor is extracted from mouse salivary glands and is ≥99% pure.

Animals and surgical procedure. Fourteen time-mated Sprague-Dawley rats were purchased from Zivic Miller Lab Inc. (Allison Park, Pa.) on day 14 of gestation. They were housed in individual cages and allowed free access to water and Purina Rat Chow (Ralston-Purina Co., St. Louis).

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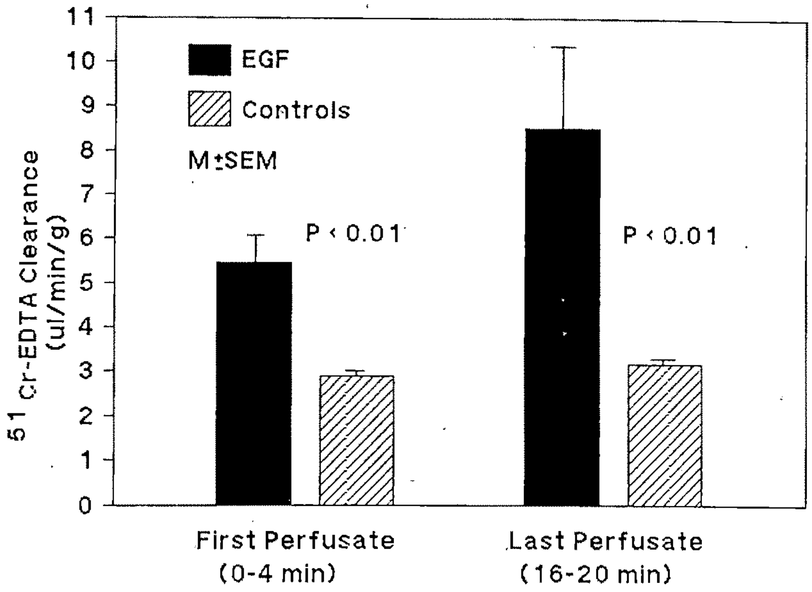

Fig. 1. Maternofetal clearances of ^{51}Cr-EDTA in epidermal growth factor versus placebo-infused animals.

Table I. Comparability of epidermal growth factor and control groups

Variable	*Epidermal growth factor*	*Control*	*Difference*
Maternal weight (gm)	418 ± 41	443 ± 33	NS
Fetal weight (gm)	4.0 ± 0.5	3.9 ± 0.2	NS
Placental weight (gm)	0.51 ± 0.04	0.49 ± 0.08	NS

NS, Not significant.

Placental perfusion. On day 20 of gestation, the dams were anesthetized with 110 mg/kg sodium thiobutabarbital, which was injected intraperitoneally. The maternal rectal temperature was maintained at 37° C through the experiment. A maternal jugular vein was cannulated for injection of tracers; a carotid artery, for blood pressure monitoring and blood sampling; and a femoral vein, for the administration of epidermal growth factor (or placebo). Maternal arterial pressure and perfusion pressure were measured with precalibrated Gould-Statham pressure transducers (Gould Inc., Cleveland) and recorded continuously on a chart recorder.

After laparotomy and hysterotomy, one of the fetuses was delivered. Modified Krebs-Ringer solution containing 118 mmol/L sodium chloride, 4.7 mmol/L potassium chloride, 1.25 mmol/L calcium chloride, 1.18 mmol/L potassium orthophosphate dihydrate, 1.18 mmol/L magnesium sulfate, 25 mmol/L sodium bicarbonate, 11 mmol/L glucose, and 3.5% dextran (40,000 d) was gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide at 37° C and pumped by a peristaltic pump (Hake-Buchler, Saddle Brook, N.J.) into the fetal circulation of the placenta, through a cannula in the umbilical artery. The perfusion rate varied from 0.28 to 0.39 ml/min between experiments but remained constant during any individual experiment. After a single pass through the placenta, perfusate effluent was collected from a cannula in the umbilical vein.

Each fetus whose placenta was perfused was removed after the vessels had been cannulated, blotted with tissue paper, and weighed to an accuracy of 0.1 mg with a Mettler H51 AR balance (Mettler Corp, Cleveland). At the end of the experiment the perfused placenta was detached from the uterine wall, and its wet weight was measured to an accuracy of 0.001 mg after fetal membranes and the umbilical cord had been dissected and discarded.

Randomization. Animals were randomized to maternal administration of epidermal growth factor ($n = 8$) or placebo ($n = 6$). Epidermal growth factor or placebo was given through the catheter, which was inserted through the maternal femoral vein. Epidermal growth factor was given as a bolus of 5 μg, followed by continuous infusion of 0.8 μg/min. This infusion was continued until the end of the experiment. The investigators were blinded as to whether the animal received epidermal growth factor in normal saline solution or placebo (saline solution alone).

Placental transfer. After 20 minutes of epidermal growth factor or placebo infusion, 40 μCi of ^{51}Cr-EDTA (500 to 800 mCi/mg) in 0.1 to 0.2 ml 0.9% sodium chloride was injected at time 0 as a bolus into the maternal circulation through a cannula in the jugular vein. At approximately 3, 13, and 25 minutes, 0.5 ml heparinized maternal blood samples were drawn from a cannula in the carotid artery for radioisotope counting. Three 4-minute collections of perfusate effluent were taken in each experiment. ^{51}Cr activity (in counts per minute) in aliquots of plasma and of perfusate effluent were counted for 10 minutes in a Packard 5330 auto-γ spectrometer (Packard Instruments Co.).

Exclusion criteria. A successful perfusion was one in which the perfused placenta was uniformly blanched (because of removal of fetal blood), and placental perfusate effluent recovery was 95% to 100% of the inflow volume. Exclusions were made before counting of plasma and perfusate samples.

Calculations. The steady-state clearance of ^{51}Cr-EDTA from maternal plasma to each perfusate sample (in microliters per minute) was defined as maternofetal clearance of ^{51}Cr-EDTA (K_{mf} ^{51}Cr-EDTA):

$$K_{mf}\ {}^{51}\text{Cr-EDTA} = (P \times F/M) \times 1000$$

where P is the ^{51}Cr concentration (counts per minute per milliliter) in perfusate effluent samples, M is the ^{51}Cr concentration (counts per minute per milliliter) in the maternal plasma at the midpoint of each 4-minute collection period, and F is the rate at which the perfusate was pumped through the placenta (milliliters per minute). To normalize for differences in placental size in different experiments, the clearance was related to the wet weight of the perfused placenta (microliters per minute per gram of placenta).

Statistics. The data are presented as mean ± SE. Differences between means in each group and over time were analyzed by means of repeated measures analysis of variance; $p < 0.05$ was considered significant.

Results

Epidermal growth factor and control groups were comparable by maternal, fetal, and placental weight at baseline (Table I).

Clearances of ^{51}Cr-EDTA were significantly affected by the infusion of epidermal growth factor; the K_{mf} ^{51}Cr-EDTA at baseline (i.e., after 20 minutes of epidermal growth factor or placebo infusion), which was calculated from the first effusate collection, was almost doubled in the epidermal growth factor group (5.45 ± 0.61 vs 2.88 ± 0.15 µl/min/gm, $p < 0.01$); it increased further in the epidermal growth factor group, to reach a value of 8.52 ± 1.84 µl/min/gm ($p < 0.001$) in the last perfusate, whereas it remained the same in the control group at 3.16 ± 0.14 µl/min/gm (not significant, compared with baseline) (Fig. 1).

Comment

Experimentally our results demonstrate that maternal-fetal ^{51}Cr-EDTA clearance is increased by maternal administration of epidermal growth factor. In adult rats epidermal growth factor is promptly removed from the circulation by hepatic sequestration and biliary secretion.[13] Thus, because of the fact that we could not infuse epidermal growth factor directly to the uterine artery for technical reasons, we used a dose of epidermal growth factor that was clearly pharmacologic both for bolus and for continuous infusion. Whether maternal serum concentrations remained elevated within the physiologic or the pharmacologic range after hepatic degradation was not determined in this pilot study. Regardless of this consideration, the mechanism by which placental permeability was increased by the exogenous growth factor requires explanation. Theoretically, augmented permeability to small molecules could be due either to an increase in "pore" number or to "pore" size.[11]

Epidermal growth factor has been demonstrated to have potent vasoconstrictor or vasodilator effects in different species, depending on the vascular bed studied.[14-16] An increase in uterine blood flow induced by epidermal growth factor, however, would have been unlikely to enhance ^{51}Cr-EDTA clearance; indeed ^{51}Cr-EDTA is a known diffusion marker that crosses the placenta according to a concentration gradient and therefore is unlikely to be affected by an increase in flow.[11]

We speculate that epidermal growth factor might be one of the regulators of placental transport of nutrients that cross the placenta by simple diffusion. This speculation would be consistent with the observation that intrauterine growth retardation in the rat increases epidermal growth factor binding to the placenta[5] (which theoretically could increase non-energy-dependent transfer of nutrients through the placenta). In contrast, in situations of excessive fetal nutrient availability such as the diabetic pregnancy in the rat, epidermal growth factor binding to placenta is reduced[4] (which theoretically could decrease non-energy-dependent transfer of nutrients through the placenta). Whether epidermal growth factor does or does not have a physiologic effect on placental function, its potent action on placental permeability, as demonstrated in this study, might provide a unique model for studying the molecular regulation of nutrient transport across the placenta.

All known biologic effects of epidermal growth factor are mediated through epidermal growth factor binding to specific cell-surface receptors.[17] Placebo (normal saline solution) did not have any measurable effect on placental permeability. We cannot, however, exclude the possibility that transforming growth factor-α, the only other protein known to bind to the epidermal growth factor receptor,[18, 19] may elicit similar changes in placental permeability.

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Effect of clomiphene citrate treatment on endometrial estrogen and progesterone receptor induction in women

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A direct adverse effect of clomiphene citrate on the endometrium has been presumed, and interference with estrogen receptor–mediated endometrial estrogen receptor and progesterone receptor induction has been implicated as the mechanism responsible for an increased incidence of luteal phase deficiency in association with clomiphene citrate treatment. To clarify the net influence of clomiphene administration on endometrial steroid receptor induction, we studied five normal ovulatory women, in both a spontaneous and clomiphene-induced (150 mg/day, cycle days 5 to 9) ovulatory cycle. From cycle day 11 blood samples were obtained daily and urinary luteinizing hormone determinations were performed twice daily. Endometrial biopsy was performed on the day of the urinary luteinizing hormone surge and again 13 days after the surge. Serum levels of follicle-stimulating hormone and luteinizing hormone were determined by immunoradiometric assay, estradiol and progesterone by radioimmunoassay, and clomiphene citrate isomer concentrations in treatment cycles by reversed-phase high-performance liquid chromatography and fluorescence detection. Total, cytosolic, and salt-extracted nuclear endometrial estrogen receptor and progesterone receptor concentrations were determined by enzyme-linked immunoassay. Serum estradiol was threefold to fivefold higher ($p < 0.05$) in clomiphene-induced than in spontaneous cycles 8 and 10 days before the luteinizing hormone surge, and progesterone was increased ($p < 0.05$) from the day of the surge to end of the cycle. Serum enclomiphene rose to plateau between 12 and 6 days before the luteinizing hormone surge (4.1 ± 0.8 ng/ml, mean ± SE, $n = 19$) and fell thereafter to <1.0 ng/ml. Zuclomiphene levels increased rapidly between 14 and 8 days before the surge (53.9 ± 2.8 ng/ml, mean ± SE, $n = 5$) and then decreased gradually but remained elevated throughout the luteal phase (29.0 ± 1.2 ng/ml, mean ± SE, $n = 33$). Late luteal endometrial histology was abnormal in one of four available treatment cycle specimens, but the endocrine characteristics and number and subcellular distribution of estrogen receptor and progesterone receptor in the abnormal cycle were not different from those of normal, in-phase cycles. Total estrogen receptor and progesterone receptor at midcycle were three to four times higher ($p < 0.01$) than in the late luteal phase in both spontaneous cycles (7.2 ± 2.3 vs 1.9 ± 0.4 pmol/mg deoxyribonucleic acid estrogen receptor and 41.3 ± 13.9 vs 13.4 ± 3.9 pmol/mg deoxyribonucleic acid progesterone receptor) and clomiphene-induced cycles (8.2 ± 2.3 vs 1.8 ± 0.8 pmol/mg deoxyribonucleic acid estrogen receptor and 40.4 ± 9.9 vs 10.1 ± 4.1 pmol/mg deoxyribonucleic acid progesterone receptor), but neither midcycle nor late luteal estrogen receptor or progesterone receptor in any category (total, cytosolic, or salt-extracted nuclear) differed from spontaneous cycle values. These data strongly suggest that clomiphene citrate treatment does not adversely affect endometrial estrogen receptor and progesterone receptor induction. (AM J OBSTET GYNECOL 1991;165:177-85.)

Key words: Clomiphene citrate, estrogen receptors, progesterone receptors, endometrium

Clomiphene citrate, a triphenylethylene compound, is the most common agent in clinical use for ovulation induction. Clomiphene citrate binds to the estrogen receptor at numerous sites throughout the reproductive system, including the hypothalamus, pituitary, ovary, endocervix, and endometrium. Its mechanism of action in ovulation induction appears to involve interference with the process of estrogen receptor replenishment at the hypothalamic level, thereby causing a central misinterpretation of circulating estrogen concentrations, the perception of a hypogonadal state, and, by altering the pattern of pulsatile gonadotropin-releasing hormone secretion, stimulation of increased pituitary gonadotropin release and enhanced follicular

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development. However, in addition to its desirable central actions, clomiphene citrate exerts the same antiestrogenic influence at sites in the periphery (some of which are undesirable and potentially counterproductive).

Clinically, luteal phase deficiency frequently complicates ovulation induction with clomiphene citrate.[1,2] Although the mechanism or mechanisms responsible remain unclear, many consider the problem to be an unavoidable consequence of competition between central and peripheral actions of the drug. Because luteal phase defects may result from inadequate progesterone secretion and/or a failure of endometrial response, levels of luteal phase progesterone production that are typically higher in clomiphene-induced than in spontaneous cycles, suggest that clomiphene may have direct adverse effects on the endometrium that predispose to luteal phase deficiency. The drug's inherently antiestrogenic nature further implies that clomiphene may interfere with estrogen induction of effective endometrial estrogen and progesterone receptor populations. Clearly, inadequate estrogen and/or progesterone receptor or abnormal receptor function could result in delayed, inadequate, or asynchronous endometrial development, but to date the net influence of clomiphene administration on endometrial steroid hormone receptor populations has not been clearly defined.

The extent of endometrial estrogen and progesterone receptor induction in cycles induced by clomiphene citrate must reflect the relative balance between two opposing influences. Although receptor induction in clomiphene-induced cycles may well be enhanced by typically elevated preovulatory estradiol levels, it may also be inhibited by a premature rise in circulating progesterone concentrations[3] and antagonized directly by the drug itself. Previous studies that we[4] and others[5-7] have conducted to examine this question have been inconclusive. Heretofore, attention has focused on the luteal phase endometrium, in which steroid receptor concentrations have normally already fallen substantially from peak values at midcycle.[8] All studies[4-7] have used conventional steroid-binding assay methods. Unfortunately, antiestrogens or their metabolites have demonstrated the capacity to effectively interfere with in vitro binding of radioligands to estrogen receptor.[9] Thus steroid-binding assay may not be entirely suitable for examinations of endometrial steroid receptor dynamics in clomiphene-treated patients.

In this study we have futher investigated the effect of clomiphene citrate on endometrial estrogen receptor and progesterone receptor populations. To avoid the influence of potentially confounding factors present in infertile populations, we studied normal ovulatory women, each of whom was examined in a spontaneous and clomiphene-induced cycle. To better evaluate the net influence of clomiphene treatment on endometrial development and steroid receptor induction, we examined both midcycle and late luteal endometrial tissue specimens in all cycles. To eliminate the possibility that administered antiestrogen might interfere with accurate receptor quantitation, we used an enzyme-linked immunoassay rather than a steroid-binding assay in determining estrogen receptor and progesterone receptor concentrations.

Material and methods

Subjects. Five normal parous women, aged 26 ± 3 years (mean ± SD), were recruited and gave informed consent for this study, which was approved by our institutional review board. Each woman had regular predictable menstrual cycles and had not used steroid contraception for at least 6 months before the study. The menstrual cycle of each participant was first screened by basal body temperature monitoring to eliminate possible gross ovulatory dysfunction.

Study design. Each woman was studied during two separate menstrual cycles. In the first cycle (control), venous blood samples were obtained on alternate days from cycle day 3 to 11. Beginning on day 11, a urine sample was obtained twice daily, the first between 11 AM and 3 PM and the second between 5 and 10 PM. Blood samples were drawn daily until 1 day after the urinary luteinizing hormone (LH) surge was identified via a rapid semiquantitative commercial enzyme immunoassay kit, Ovustick (Monoclonal Antibodies, Inc., Mountain View, Calif.) that uses the first international standard for menopausal gonadotropins. Thereafter, urine sampling was discontinued and blood sampling returned to alternate days until the onset of menses. An endometrial biopsy speciman was obtained from the uterine fundus with a disposable aspiration cannula (Pipelle, Unimar, Wilton, Conn.) on the day of the urinary LH surge and again 13 days later. In the second cycle blood and urine sampling and endometrial biopsy were carried out in an identical fashion, and 150 mg of clomiphene citrate (Clomid, Merrell, Cincinnati) was self-administered in a single daily dose on cycle days 5 through 9.

Venous blood samples were allowed to clot, and the separated serum was frozen at −20° C until it was assayed for estradiol, progesterone, follicle-stimulating hormone (FSH), LH, and clomiphene isomers. Representative samples of each endometrial tissue specimen were fixed in 10% formalin; the remainder of each specimen was placed in a sealable plastic envelope, immediately snap-frozen by immersion in liquid nitrogen, and stored at −80° C for determination of cytosolic and salt-extracted nuclear endometrial estrogen and progesterone receptor concentrations. Histologic sections were examined by three independent gynecologic pathologists, all using the criteria of Noyes et al.[10] and without knowledge of the experimental conditions. Each late luteal phase biopsy specimen was assigned a histologic date encompassing a 2-day interval and interpreted according to the most advanced day; the mean result was judged with reference to both the serum LH surge and onset of menses. Endometrial specimens that were ≥3 days out of phase were considered abnormal and consistent with the diagnosis of luteal phase defect.

Hormone, clomiphene, and receptor assays. Serum estradiol and progesterone concentrations were measured by specific radioimmunoassay; concentrations of FSH and LH were measured by immunoradiometric assay with commercial assay kits (Diagnostic Products, Los Angeles). To reduce interassay variability, all serum samples from the control and clomiphene treatment cycles of each individual woman were assayed together for each hormone. The intraassay and interassay coefficients of variation were <10% in all four assay systems. Detection limits were 0.12 and 0.2 mIU/ml for LH and FSH, respectively, and 8.0 pg/ml and 0.5 ng/ml for estradiol and progesterone, respectively. Gonadotropin values were expressed in international units of the first international reference preparation 68/40 for LH and the second international reference preparation of human menopausal gonadotropin for FSH. Serum concentrations of enclomiphene and zuclomiphene isomers in treatment cycles were determined by methods previously described in detail.[11] Briefly, samples were extracted with tert-butyl methyl ether, and the drug and its metabolites were separated by reversed-phase high-performance liquid chromatography. The eluent was fed into a braided or woven, cylindric Teflon reaction coil into which a low-energy mercury lamp had been inserted. The result was a photoinduced stilbene-to-phenanthrene oxidation that yielded highly fluorescent analytes that were then quantified with fluorescence spectrophotometry. The assays were performed by Hazelton Laboratories (Madison, Wis.) in affiliation with Serono Laboratories (Norwell, Mass.). Intraassay and interassay coefficients of variation were <10% for both the enclomiphene and zuclomiphene isomers.

Frozen endometrial tissue specimens were thawed on ice and homogenized (1:10, wt/vol) in TEDG-Mo buffer (0.05 mol/L Tris hydrochloride, 1.5 mmol/L sodium ethylenediaminetetraacetic acid, 0.5 mmol/L dithiothreitol, 10% glycerol, and 10 mmol/L sodium molybdate; pH 7.5) with two bursts (10 seconds) of a precooled Polytron (Brinkman Instruments, Westbury, N.Y.) at a setting of 5 with a 10-second cooling interval. Tissue homogenates and all reagents were maintained at 0° to 4° C during processing and assay. Homogenates were centrifuged at 800 *g* for 10 minutes to provide a crude nuclear pellet. To prepare the cytosol fraction, the supernatant was removed and centrifuged at 40,000 *g* for 20 minutes; it was then diluted with TEDG-Mo buffer to yield a protein concentration of 0.5 to 1.0 mg/ml as determined by the method of Lowry et al.[12] Crude nuclear pellets were washed once with TEDG-Mo buffer (5 ml), and a nuclear extract was obtained by resuspending pellets in hypertonic buffer (0.6 mol/L potassium chloride–TEDG-Mo buffer) and incubating them for 60 minutes. Before recentrifugation (40,000 *g* for 20 minutes), nuclear extracts were diluted 1:1 with TEDG-Mo buffer to yield a protein concentration in a range from 0.5 to 1.0 mg/ml. Estrogen receptor and progesterone receptor enzyme-linked immunoassay kits (Abbott Laboratories, North Chicago) were obtained, and the assays were conducted according to the manufacturer's specifications. Endometrial estrogen and progesterone receptor concentrations were expressed in picomoles per milligram of deoxyribonucleic acid after quantitation of tissue deoxyribonucleic acid content by the method of Burton.[13]

Data analysis. Hormone data for all cycles and drug data from treatment cycles were synchronized to the day of the serum LH peak (LH + 0), and significant differences in hormone concentrations between the control and clomiphene treatment cycles were determined by two-way analysis of variance (day of cycle and experimental groups) for repeated measures. Results of histologic endometrial dating were compared with the McNemar test. Receptor concentrations within and between control and treatment cycles were compared with paired *t* tests; $p < 0.05$ was considered significant.

Results

Clinical findings. Each of the menstrual cycles of all five women was ovulatory, as judged by typical biphasic basal body temperature patterns, the identification of an LH surge in both urine and serum, a postovulatory rise in serum progesterone concentrations, and the finding of secretory endometrium in the second biopsy specimen obtained in each of the studied cycles (both control and clomiphene treatment cycles. A urinary LH surge was identified in all cycles and correlated well with that in serum. In all women peak serum LH concentrations occurred on or 1 day after the day of urinary LH surge onset. As a result, midcycle endometrial biopsies in both control and clomiphene-induced cycles were performed on LH − 1 or LH + 0, and late luteal endometrial biopsy specimens were uniformly obtained on LH + 12 or LH + 13 in 9 of 10 study cycles. One patient experienced onset of menses before a scheduled late luteal biopsy after clomiphene treatment. Therefore, data derived from treatment cycle LH + 13 tissue

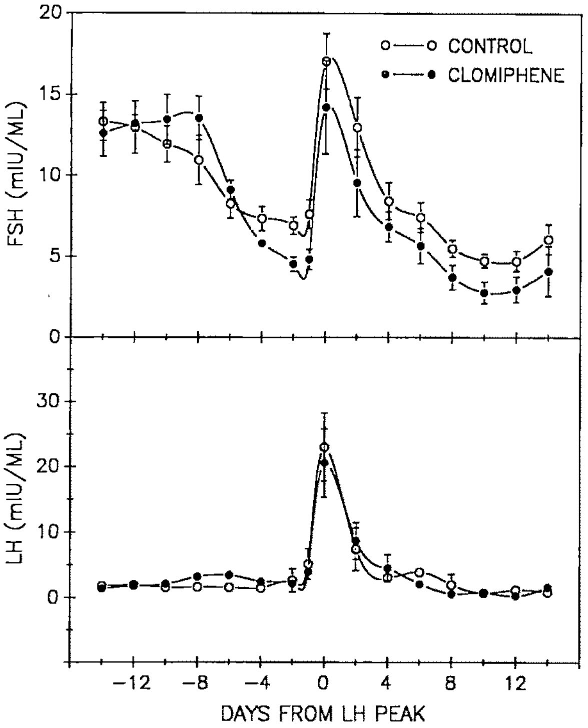

Fig. 1. Serum FSH and LH concentrations (mean ± SE) in five women during a spontaneous cycle (control) and a cycle in which they received clomiphene citrate (150 mg/d, cycle days 5 to 9). FSH values are expressed in terms of second international reference preparation human menopausal gonadotropin and those of LH in terms of the first international reference preparation 68/40.

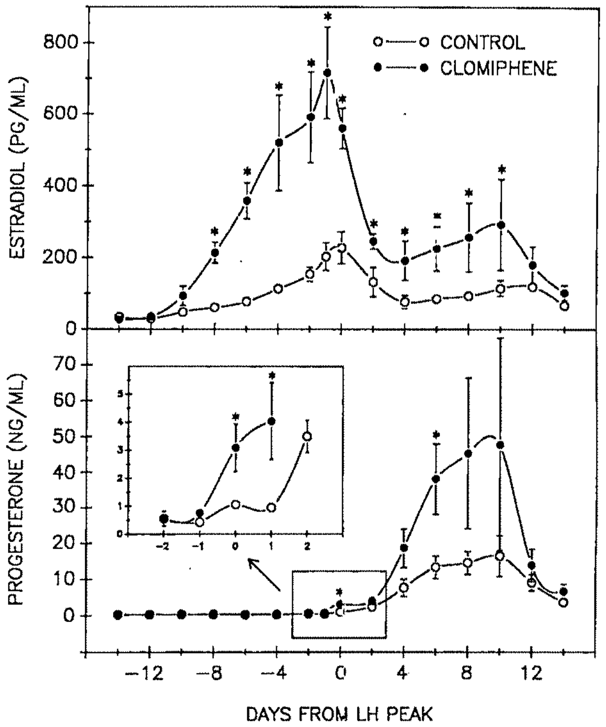

Fig. 2. Serum estradiol and progesterone concentrations (mean ± SE) in five women during a spontaneous cycle (control) and during a cycle in which they received clomiphene citrate (150 mg/day, cycle days 5 to 9). *Asterisk*, $p < 0.05$.

specimens reflect results obtained in four of the five women studied. Overall cycle length, day of LH surge, and luteal phase duration are summarized in Table I. None of these paramters in clomiphene treatment cycles was significantly different from corresponding control cycle values.

Hormone data. Serum LH and FSH concentrations in the control and clomiphene treatment cycles of the five women are shown in Fig. 1. Although midfollicular-phase FSH levels tended to be higher and luteal phase concentrations lower in clomiphene cycles, the differences were not significant. Similarly, LH levels throughout the cycle of clomiphene administration were not different from those observed in control cycles. Serum concentrations of estradiol and progesterone in control cycles and after clomiphene treatment are illustrated in Fig. 2. As expected, serum estradiol levels in clomiphene cycles were significantly higher ($p < 0.05$) than corresponding control cycle values. Estradiol concentrations remained significantly elevated throughout the interval from day LH − 8 to day LH + 10 in clomiphene cycles; levels in the follicular phase were threefold to fivefold greater than they were without clomiphene treatment. Serum progesterone concentrations were also significantly higher ($p < 0.05$) after clomiphene administration. This difference first became apparent in the periovulatory phase; significantly increased progesterone levels were observed on days LH + 0 and LH + 1. Mean serum progesterone level remained elevated throughout the luteal phase but to a widely varying extent among patients; concentrations in one individual rose as high as 166 ng/ml.

Clomiphene isomers. Serum concentrations of enclomiphene and zuclomiphene in treatment cycles are shown in Fig. 3. Enclomiphene levels rose abruptly to a plateau (4.1 ± 0.8 ng/ml, mean ± SE, $n = 19$) between day LH − 12 and day LH − 6. Thereafter, concentrations fell equally as abruptly to <1.0 ng/ml, where they remained for the rest of the cycle. In contrast, levels of zuclomiphene increased rapidly from day LH − 14 to peak on day LH − 8 (53.9 ± 2.8 ng/ml, mean ± SE, $n = 5$) and then very gradually fell until onset of the subsequent menses. The overall mean luteal phase concentration of zuclomiphene was 29.0 ± 1.2 ng/ml (mean ± SE, $n = 33$).

Receptor concentrations. Endometrial estrogen receptor concentrations are shown in Fig. 4. As we had anticipated, midcycle (day LH + 0) levels of total, cytosolic, and salt-extracted nuclear estrogen receptor were significantly higher ($p < 0.01$) than late luteal

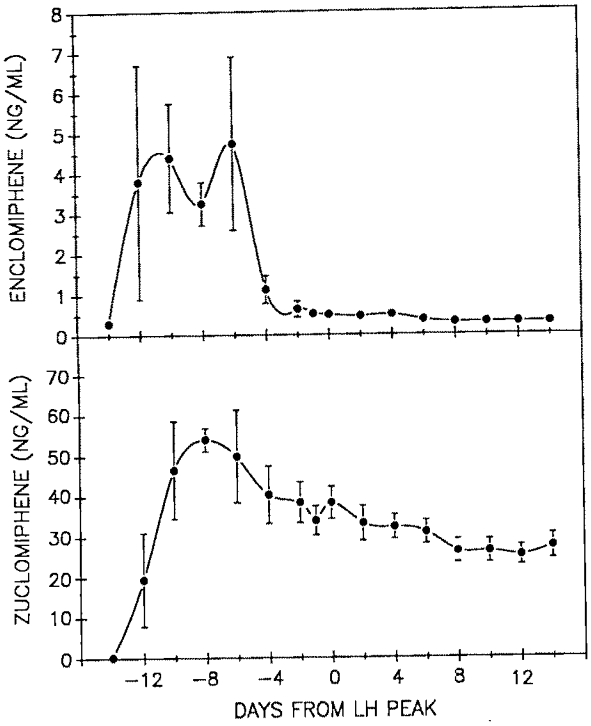

Fig. 3. Serum enclomiphene and zuclomiphene concentrations (mean ± SE) in five women during a cycle in which they received clomiphene citrate (150 mg/day, cycle days 5 to 9).

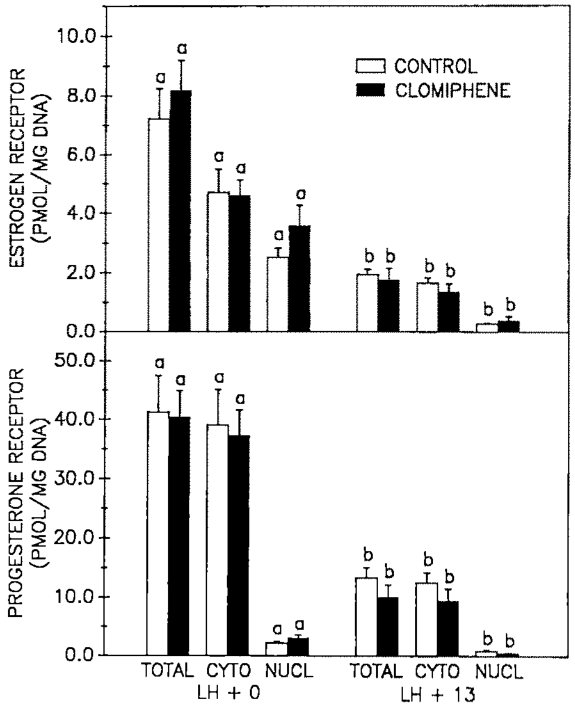

Fig. 4. Endometrial concentrations (mean ± SE) of *total*, cytosolic *(cyto)*, and salt-extracted nuclear *(nucl)* estrogen and progesterone receptor in five women during a spontaneous cycle and during a cycle in which they received clomiphene citrate (150 mg/day, cycle days 5 to 9). In each class *(total, cyto,* and *nucl) bars with different superscripts* are different ($p < 0.01$).

phase (LH + 13) values in both control and clomiphene treatment cycles. However, despite the significant sustained increase in circulating estradiol levels associated with clomiphene administration, neither midcycle nor late luteal estrogen receptor concentrations in any of the three categories differed significantly from control cycle values. In both cycles the proportion of total estrogen receptor recovered from nuclear extracts was significantly greater ($p < 0.01$) in the LH + 0 tissue specimens than in the LH + 13 biopsy specimens, but neither the midcycle luteal values nor the late luteal values were altered by the clomiphene treatment.

Immunoassayable endometrial progesterone receptor concentrations are also illustrated in Fig. 4. As was true of estrogen receptor, total, cytosolic, and salt-extracted nuclear progesterone receptor on day LH + 0 were all significantly elevated ($p < 0.01$) when compared with day LH + 13 values in both control and clomiphene treatment cycles. Again, however, both midcycle and late luteal progesterone receptor concentrations in all three categories remained unchanged after clomiphene administration. The proportion of total endometrial progesterone receptor residing within the nuclear fraction was relatively low in all tissue specimens (6.5% ± 2.6%, mean ± SE, $n = 19$) and did not vary with phase of cycle or clomiphene treatment. Despite the fact that mean periovulatory progesterone levels were significantly higher in clomiphene cycles (Fig. 2), there was no associated significant shift in the overall mean percent nuclear progesterone receptor observed between the two cycles in day LH + 0 specimens. There was, however, a tendency toward increased percent nuclear progesterone receptor in clomiphene cycle day LH + 0 tissues in those patients who had the greatest relative increases in periovulatory progesterone over the respective control cycle values.

Endometrial histology. Late luteal phase endometrial histology revealed no significant difference between control and clomiphene treatment cycles overall. All control cycle biopsy specimens and three of the four treatment cycle specimens were judged normal, whether they were evaluated with reference to the serum LH surge or to the onset of the next menses. The treatment cycle biopsy specimen in one patient was abnormal because it exhibited a maturation delay of 4 and 5 days relative to onset of menses and LH surge, respectively, but neither the endocrine characteristics nor the number and subcellular distribution of midcycle or late luteal phase endometrial estrogen or progesterone receptor in this abnormal cycle were significantly different from those values in the remaining three treatment cycles in which the histology was normal.

Table I. Cycle characteristics before and during clomiphene citrate treatment (mean ± SEM)

Characteristic	*Cycle*	
	Control	*Clomiphene*
Cycle length (days)	30.8 ± 1.3	29.8 ± 0.9
LH surge (cycle day)	17.2 ± 1.2	16.4 ± 0.8
Luteal phase duration (days)	13.6 ± 0.2	13.4 ± 0.4

Comment

Clomiphene citrate has been implicated as a cause of luteal phase deficiency and, paradoxically, also is advocated for treatment of the same disorder. Its therapeutic potential derives from luteal phase steroid hormone production that is consistently greater in clomiphene-induced than in spontaneous ovulatory cycles. The higher circulating steroid levels almost certainly result from a drug-induced increase in gonadotropin secretion, improved folliculogenesis in the single dominant follicle and/or the larger cohort of follicles recruited to participate in the treatment cycle,[14] and a subsequent increase in overall luteal cell mass. Although the drug's extended half-life may alternatively afford it the opportunity to influence corpus luteum steroidogenesis more directly, available evidence indicates that such effects, if they are exerted at all, are inhibitory in nature.[15] However, the ability of clomiphene to antagonize estrogen actions in the periphery may at the same time prevent an appropriate endometrial response to normal or even increased steroid concentrations. Because normal secretory endometrial maturation requires the combined actions of estrogen and progesterone and because both steroids exert their effects through specific estrogen-induced endometrial receptors, direct interference with estrogen induction of effective endometrial estrogen and progesterone receptor populations is one mechanism that might explain the increased incidence of luteal phase deficiency associated with clomiphene use.[1,2]

Clomiphene treatment typically increases late follicular phase serum estradiol and progesterone concentrations; both may exert significant, if opposite, influence on endometrial steroid receptor induction and thus may modify any direct antiestrogenic actions of clomiphene itself. The effect of these many competing direct and indirect clomiphene-induced changes has not been clear. Clomiphene has been shown to reduce concentrations of endometrial estrogen and progesterone receptor induced by estrogen replacement in postmenopausal women. Moreover, decreased concentrations of both receptors have been identified in late luteal phase endometrium after clomiphene induction of ovulation,[5] after continuous luteal phase clomiphene administration in normal women,[4] and in tissue specimens obtained at the time of oocyte retrieval after combined treatment with clomiphene, human menopausal gonadotropins, and human chorionic gonadotropin for in vitro fertilization.[6] Together, these data suggest that clomiphene does interfere with the induction and/or maintenance of effective endometrial steroid receptor populations. Other studies, however, deny that clomiphene has an adverse effect on the endometrium. A recent report that periimplantation phase nuclear estrogen and progesterone receptor levels and binding affinity in normal ovulatory women were no different after both low-dose (50 mg) and high-dose (150 mg) clomiphene treatment than in spontaneous cycles is of particular interest.[7] Interpretation of these conflicting data has been confounded by diversity in study populations and methodologic differences and is now further complicated by recent evidence that conventional steroid-binding assay methods used in these studies may yield inaccurate estrogen receptor determinations in tissues exposed to antiestrogens in vivo.

To clarify the effect of clomiphene on endometrial estrogen and progesterone receptor induction, we elected to examine tissue specimens that would reflect both the peak (midcycle) and nadir (late luteal phase) in receptor populations. We used a recently developed enzyme-linked immunoassay to measure total, cytosolic, and salt-extracted nuclear estrogen and progesterone receptor concentrations in endometria derived from both spontaneous and clomiphene-induced cycles in the same patients. The enzyme-linked immunoassay is based on the recognition of two distinct antigenic determinants in the receptor molecule that are distinct from the hormone-binding region and that require the presence of an intact receptor molecule for detection. The method is therefore independent of the state of receptor occupancy and applies equally well to receptor quantitation in both cytosol and nuclear fractions of tissue homogenates. Enzyme-linked immunoassay determinations of cytosolic and nuclear estrogen and progesterone receptor generally correlate well with data generated by steroid-binding assay in both normal endometrium[16] and in breast cancer tissue[9] and have been demonstrated to accurately predict the endocrine dependence of human breast cancers. However, only immunoassay detected significant levels of cytosolic estrogen receptor in breast tumor tissue from patients under treatment with tamoxifen, a potent antiestrogen[9]; steroid-binding assay failed to detect estrogen receptor in these specimens. The mechanism responsible for this discrepancy is not yet clear, but these data demonstrate the potential for steroid-binding assay to underestimate estrogen receptor in tissues of patients treated with antiestrogens.

The increase in follicular phase estradiol levels observed in treatment cycles was striking and remarkable in the absence of a distinct rise in gonadotropin secretion. Mean FSH and LH levels were indeed higher than corresponding control values during the interval of clomiphene administration, but the differences were not statistically significant, probably because the rise in each was subtle and the sample size small. The possibility remains that clomiphene administration may impair the metabolic clearance of estradiol or may result in the release of an altered form of FSH that has enhanced biologic activity, but there is no evidence to indicate that clomiphene might stimulate ovarian steroidogenesis directly.[15] The increase in estradiol production after clomiphene treatment probably reflects the recruitment of additional follicles more than increased estrogen synthesis in the dominant follicle already selected.[14] Regrettably, however, we did not monitor follicular development with serial ultrasonography in this study, and we acknowledge that the mechanism responsible for the marked rise in estradiol levels requires additional investigation.

As anticipated, the concentrations of total, cytosolic, and salt-extracted nuclear estrogen and progesterone receptor were markedly higher at midcycle than during the late luteal phase. These findings are consistent with earlier studies[8] and reflect the largely unopposed influence of estrogen on estrogen and progesterone receptor induction during the follicular phase of the menstrual cycle. Despite an approximately threefold increase in circulating estradiol levels after clomiphene administration, no significant net change in the concentration of estrogen or progesterone receptor in any category was observed in treatment cycles. The failure of elevated estradiol to induce increased concentrations of endometrial estrogen and progesterone receptor at midcycle may result from the inhibition of circulating clomiphene and/or the premature periovulatory rise in serum progesterone, because progesterone inhibits estrogen receptor production and, indirectly, the induction of its own receptor. Alternatively, already maximal concentrations of endometrial estrogen and progesterone receptor may be induced by normal ovulatory threshold levels of estradiol, and elevated estrogens may, conversely, buffer an otherwise inevitable decline in receptor levels caused by clomiphene and/or elevated progesterone. Our data are in general agreement with those of Hecht et al.[7] in a recent study of midluteal phase endometrium that uses a similar design. Their somewhat lower absolute levels of receptor may be attributed to the use of crude nuclear suspensions and elevated incubation temperatures (which are characteristic of steroid-binding assay) that may lead to underestimation of nuclear steroid receptor content, in contrast to our use of high-salt nuclear extracts and enzyme-linked immunoassay. Although we observed no change in the number and subcellular distribution of estrogen and progesterone receptor in whole endometrium after clomiphene treatment, a shift in the distribution of estrogen and progesterone receptor between the stromal and glandular compartments may have occurred and gone unrecognized. Complex and dyssynchronous fluctuations in the secretory phase progesterone receptor content of endometrial glands and stroma have recently been demonstrated.[17] Such changes would not be detected by enzyme-linked immunoassay and could be demonstrated only by an immunocytochemical approach to steroid receptor detection.

The late luteal phase endometrial histology was normal in three of four available treatment cycle specimens, exhibited a clinically significant maturation delay consistent with luteal phase deficiency in one individual, and was not evaluated in another patient because of the early onset of menses. The endocrine characteristics and number and subcellular distribution of endometrial estrogen and progesterone receptor in the abnormal cycle were indistinguishable from those of in-phase cycles. Although the number of individuals studied is small, these data clearly argue against drug-induced disruption of endometrial steroid receptor induction as the mechanism of luteal phase deficiency associated with the administration of clomiphene citrate. Nevertheless, the clincial relevance of our findings in normal ovulatory women may be questioned because numerous studies of endometrial steroid receptor levels have failed to establish inadequate or dysfunctional receptors as a cause of luteal phase deficiency and because the link drawn between clomiphene treatment and the increased risk of luteal phase inadequacy derives from observations made in anovulatory infertile patients undergoing long-term therapy.

The widely varying estrogen and progesterone receptor content of endometrial tissue derived from infertile women with spontaneous luteal phase defects probably reflects only the many and varied mechanisms that may cause luteal phase inadequacy, as well as the unavoidable study of etiologically diverse populations within and between such studies. In any case the lack of a characteristic endometrial steroid receptor profile in luteal phase deficiency does not exclude altered receptor dynamics as a cause of the disorder. However, the relative endocrine impact of experimental acute clomiphene administration in normal women may indeed significantly differ from that in anovulatory individuals who receive continuous treatment. The extent to which the estrogen agonistic and antagonistic actions of clomiphene isomers are expressed may vary with dose, duration of therapy, and the differing endocrine milieus of normal and anovulatory women. The extended half-life of clomiphene and its corresponding potential for significant systemic accumulation have been demonstrated. Even in our isolated treatment cycle, we observed a striking, sustained elevation of serum zuclomiphene throughout the luteal phase. Circulating concentrations that are higher still almost certainly exist in patients who fail to conceive promptly and who therefore continue treatment for extended periods of time. Therefore dose- and time-dependent adverse effects of clomiphene on endometrial steroid receptor populations cannot be excluded, in spite of the use of high-dose clomiphene in this study and in other studies that have failed to demonstrate such an effect.

If clomiphene treatment does, in fact, predispose to an increased risk of luteal phase deficiency, mechanisms that are unrelated to endometrial steroid receptor concentrations and/or function are likely to be involved. These mechanisms may be unique to clomiphene cycles or they may be similar to those that operate in women with spontaneous luteal phase defects. Direct adverse endometrial effects of clomiphene that operate through post-receptor mechanisms remain a possibility. The occasional marked elevation of luteal phase estradiol that follows clomiphene-induced ovulation may directly interfere with endometrial decidualization,[18] and there is evidence that clomiphene may directly inhibit corpus luteum steroidogenesis.[15] Second, inadequate luteal function is often only a natural consequence of similarly inadequate folliculogenesis; luteal phase deficiency consistently accompanies follicular phase FSH deficiency that is spontaneous or experimentally induced. Because clomiphene induction of ovulation is an empiric incremental titration in which success depends on the extent of induced pituitary FSH release, clomiphene-associated luteal phase deficiency may at times reflect only inadequate treatment rather than a specific deleterious action of the drug itself. An abnormally increased follicular phase LH pulse frequency is a subtle aberration of gonadotropin secretion that is also implicated in the pathogenesis of luteal phase defects.[19] Interestingly, clomiphene appears to induce a similar increase in LH pulse frequency which presumably reflects an increase in pulsatile hypothalamic gonadotropin-releasing hormone secretion.[20] Excess clomiphene, whether it results from frank overdosage or accumulation after long-term administration, may provoke an abnormally rapid pattern of gonadotropin secretion that predisposes to inadequate corpus luteum function. Regardless of the mechanism involved, the potential for luteal phase deficiency to accompany either too much or too little drug suggests that the effective therapeutic range for clomiphene may be narrower than that previously appreciated, at least in some individuals.

In summary, clomiphene citrate treatment (150 mg) did not affect concentrations of immunoassayable midcycle or late luteal phase endometrial estrogen receptor and progesterone receptor in normal ovulatory women. Although an adverse effect of low-dose or long-term clomiphene citrate administration cannot be excluded, these data refute a drug-induced disruption of endometrial steroid receptor induction as the mechanism of luteal phase deficiency associated with clomiphene citrate.

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The effect of the inhibition of prostaglandin synthesis on renal blood flow in fetal sheep

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Treatment with prostaglandin synthesis inhibitors has been associated with oligohydramnios in the fetus. The presumed mechanism is a reduction in fetal renal blood flow. We examined the effect of meclofenamate administration on renal blood flow in chronically catheterized fetal sheep during normoxia and during moderate and severe hypoxia. Ten fetal sheep were made hypoxic twice at least 4 days after surgery: once in the presence and once in the absence of meclofenamate infusion. Renal blood flow and combined ventricular output were measured with radioactive microspheres. Prostaglandin synthesis blockade with meclofenamate caused no significant change in blood pressure, combined ventricular output, renal blood flow, or renal vascular resistance in either the normoxic or hypoxic animals. These data challenge the contention that prostaglandin activity protects the renal vascular bed of the fetus from vasoconstriction during hypoxia and they also do not support the hypothesis that prostaglandin synthesis inhibition causes oligohydramnios through reduction of fetal renal blood flow. (AM J OBSTET GYNECOL 1991;165:185-90.)

Key words: Fetal renal blood flow, prostaglandin synthetase inhibitors, meclofenamate, fetal hypoxia, fetal sheep

Prostaglandin synthetase inhibitors have emerged as useful agents in the therapy of preterm labor.[1-3] In spite of their tocolytic efficacy and ease of administration, widespread clinical use has been limited by fetal side effects. Oligohydramnios has developed in as many as 50% of patients treated with prostaglandin synthetase inhibitors.[4-5]

Prostaglandin synthetase inhibitors are known to decrease fetal urine output[6] and thereby lead to oligo-

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Table I. Effect of meclofenamate on fetal ascending aortic blood gas values, pH, heart rate, and blood pressure

	Control	*Meclofenamate*	*Meclofenamate % control*
Oxygen pressure (mm Hg)	22 ± 1	22 ± 1	104 ± 5
Oxygen content (mmol/L)	2.8 ± 0.2	2.4 ± 0.3	85 ± 5
Carbon dioxide pressure (mm Hg)	50 ± 1	52 ± 1	103 ± 2
pH	7.40 ± 0.01	7.33 ± 0.03	99 ± 1
Heart rate (beats/min)	180 ± 5	206 ± 11	114 ± 5
Blood pressure	59 ± 4	56 ± 4	96 ± 5

Mean ± SEM; n = 10.

Control vs meclofenamate and meclofenamate % control vs. 100%; no statistically significant differences.

hydramnios. This decrease in fetal urine output may result from either a decrease in fetal renal blood flow or an increase in tubular free water reabsorption. Some have reported that prostaglandin synthetase inhibitors reduce basal renal blood flow.[7] Others, however, demonstrated a decrease in renal blood flow only during status of high circulating norepinephrine, α-adrenergic neural stimuli, or elevated angiotensin II, as seen with fetal hypoxia or hypotension.[8-11]

To test the hypothesis that prostaglandin inhibition causes a decrease in fetal renal blood flow, we assessed the effect of prolonged fetal administration of meclofenamate, a prostaglandin synthesis inhibitor, on basal renal blood flow and on renal blood flow during moderate and severe hypoxia in a chronically catheterized sheep model.

Material and methods

We studied 10 fetal sheep at 125 to 130 days' gestation, products of Western Breed ewes and Black face rams. The animals were studied in accordance with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society.

Surgical preparation. Fasted pregnant ewes were sedated with intramuscular ketamine hydrochloride (8 mg/kg), intubated, and given halothane to induce general anesthesia. Polyvinyl catheters of 2.3 mm external diameter were inserted into the maternal femoral vein and artery and advanced into the inferior vena cava and aorta. The uterus was exposed through a ventral midline incision. The fetal hind limbs were delivered through a small hysterotomy and catheters with a 1.2 mm external diameter were inserted into the pedal artery and vein and advanced into the inferior vena cava and aorta. Through a separate hysterotomy, both front limbs were delivered and catheters of 1.2 mm external diameter were inserted into the axillary artery and advanced into the ascending aorta. An open-ended catheter was sutured into the intrauterine cavity and filled with saline solution for measurement of amniotic fluid pressure. The vascular catheters were filled with heparin sulfate solution (1000 U/ml). Intramuscular dihydrostreptomycin (1.25 gm), and intravenous procaine penicillin G (1 million U) were administered to the ewe on the day of surgery and on each postoperative day.

Measurements. Maternal arterial and fetal arterial blood pressure levels and amniotic fluid pressure were recorded continuously with pressure transducers (P23Db, Statham, Oxnard, Calif.). Fetal blood pressure was corrected for amniotic fluid pressure, which was used as the zero reference pressure. Maternal heart rate was determined from the tracing of pulsatile blood pressure. Fetal heart rate was recorded with a cardiotachometer. Blood values, pH, oxygen saturation and hemoglobin concentration were measured on a blood gas monitor (HM 73, Radiometer, Copenhagen) and OSM2 hemoximeter (Radiometer).

Fetal combined ventricular output and renal blood flow values were determined with the radionuclide-labeled microsphere method.[12] Microspheres 15 μm in diameter were injected into the inferior vena cava while reference blood samples were withdrawn from the ascending and distal aorta at a rate of 3.8 ml/min. The microspheres were labeled with the following radionuclides: gadolinium 153, cobalt 57, indium 114, chromium 51, stannum 113, strontium 85, niobium 95, zinc 65, or manganese 54 (3-M Company, St. Paul, Minnesota). After each microsphere injection, withdrawn fetal blood was replaced with 15 ml of heparinized maternal blood collected at the beginning of the experiment.

At the conclusion of the study, the ewes were killed with a rapid intravenous injection of sodium pentobarbital and 3 mol/L potassium chloride. The fetus was removed and weighed. The fetal organs were dissected, weighed, ashed, and powdered as previously described.[12] Radioactivity was counted in a 1000-channel computerized pulse height analyzer (Ino-Tech Inc., Wisconsin).

Vascular resistance estimation was obtained by dividing the arterial-venous pressure difference by the renal blood flow. Central venous pressure was not measured in these animals but assumed to be constant at 3 mm Hg as shown by previous studies.[13]

Experimental protocol. The animals were studied 3 to 6 days after surgery. Data were recorded from the mother and the fetus while the ewe stood quietly in a study cage. Each fetus was studied twice. One of the

Table II. Effects of meclofenamate infusion on fetal combined ventricular output, renal blood flow, and renal vascular resistance

	Control	*Meclofenamate*	*Meclofenamate % control*
Cardiac output (ml · $min^{-1}kg^{-1}$)	460 ± 50	429 ± 54	93 ± 6
Renal blood flow (ml · min^{-1} · 100 gm^{-1})	216 ± 31	204 ± 24	87 ± 8
% CVO perfusing the kidneys	3.1 ± 0.3	3.0 ± 0.3	90 ± 5
Renal vascular resistance (mm Hg · min · 100 gm · ml^{-1})	0.30 ± 0.05	0.27 ± 0.02	118 ± 15

CVO, Central venous oxygen.
Mean ± SEM; n = 10.
Control vs. meclofenamate and meclofenamate percentage of control vs 100%; no statistically significant differences.

studies consisted of measuring the fetal response to hypoxia alone, whereas the other study consisted of measuring the fetal response to hypoxia after pretreatment with a prolonged infusion of meclofenamate. To eliminate the possible effect of experimental deterioration over time the order of studies was randomly assigned. In two animals hypoxia alone was preceded by infusion of the carrier solution without the meclofenamate to exclude an effect of the carrier as well as the volume load on fetal cardiac output and blood flow distribution.

The hypoxic study consisted of a control period of 20 minutes followed by a period of hypoxia. The duration of hypoxia necessary to cause severe hypoxia (defined as Ca_{O_2} <1.0 mml^{-1}) varied from animal to animal (range, 20 to 35 minutes). Fetal hypoxia was induced by placing the head of the ewe in a clear plastic bag and administering an oxygen-poor gas mixture of air, nitrogen, and carbon dioxide. The maternal P_{CO_2} was kept constant by adjusting the amount of inspired carbon dioxide. During the experiment, maternal blood and amniotic fluid pressure and heart rate and fetal blood pressure and heart rate were measured continuously. Fetal cardiac output and blood flow distribution were measured at the end of the control period and three times during the hypoxia period when the fetal ascending aortic oxygen content was measured to be between 1.5 and 1.8 mml^{-1}, 1.0, and 1.5 mml^{-1} and <1.0 mml^{-1}. Blood gas values, pH, oxygen saturation, and hemoglobin measurements were done on blood samples from the maternal aorta and from the fetal ascending aorta and inferior vena cava before and after each blood flow determination.

The meclofenamate study consisted of a control period of 20 minutes, a drug infusion period of 4 hours, and a period of hypoxia. The duration of hypoxia necessary to cause severe hypoxia (defined as Ca_{O_2} <1.0 mm^{-1}) varied from animal to animal and ranged between 15 and 35 minutes. Meclofenamate (50 mg) was dissolved with 2 ml of 0.715% sodium carbonate and diluted with 40 ml of distilled water and 60 ml of normal saline solution. Meclofenamate was administered with an initial bolus of 20 mg (approximately 6 mg/kg fetal weight) given over 10 minutes, followed by a 4-hour infusion at a dose of 1.15 mg/hr (approximately 0.3 mg/kg fetal weight).

During the experiment, maternal blood pressure, amniotic fluid pressure, and heart rate and fetal blood pressure and heart rate were measured continuously. Fetal cardiac output and blood flow distribution were measured at the end of the control period, after the 4 hours of meclofenamate infusion, and three times during the hypoxia period when the fetal oxygen content was measured as between 1.5 and 1.8 mml^{-1}, 1.0 and 1.5 mml^{-1}, and <1.0 mml^{-1}. Blood gas values, pH, oxygen saturation, and hemoglobin measurements were done on blood samples from the maternal aorta and from the fetal ascending aorta and inferior vena cava before and after each blood flow determination.

Analysis of data. To reduce the effect of interanimal variability the data are also expressed as percentage of control, where each animal serves as its own control.

All values in text, tables, and figures are means ± SEM. Statistical analysis was performed with one-way analysis of variance. When the data were found to be asymmetric the Mann-Whitney test was used. A difference was considered significant when the p value was less than 0.05.

Results

Fetal response to meclofenamate infusion. Four-hour infusion of meclofenamate did not produce a significant change in blood gas values, blood pressure, combined ventricular output, renal blood flow, or calculated renal vascular resistance as shown in Tables I and II. To verify that interanimal variability had not masked a positive result, each animal was used as its own control. Renal blood flow expressed as percentage of control and percentage of cardiac output also failed to show a significant change after meclofenamate infusion.

Response to hypoxemia. The administration of

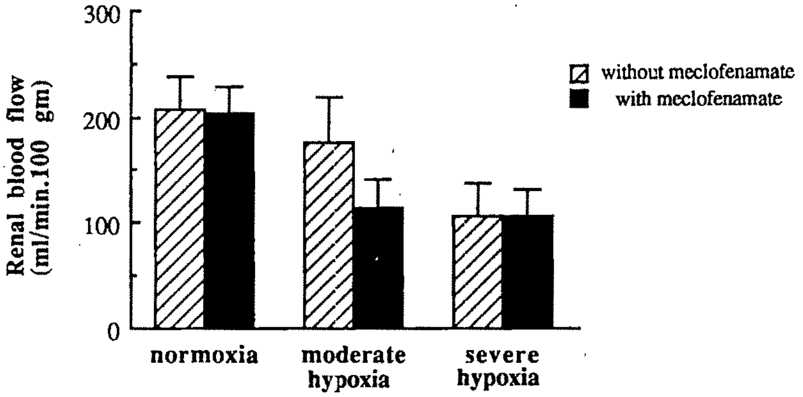

Fig. 1. Renal blood flow response to hypoxia alone and to hypoxia with meclofenamate. Meclofenamate treatment did not cause a significant change in blood flow as compared with hypoxia alone. Mean ± SEM.

Table III. Fetal response to moderate and severe hypoxia with and without pretreatment with meclofenamate

	Normoxia		*Moderate hypoxia*		*Severe hypoxia*	
	Alone	*Before meclofenamate*	*Alone*	*With meclofenamate*	*Alone*	*With meclofenamate*
Oxygen pressure (mm Hg)	24 ± 3	21 ± 2	14 ± 2*	15 ± 1*	13 ± 2	12 ± 1*
Oxygen content (mM/L)	2.8 ± 0.3	2.8 ± 0.3	1.15 ± 0.04*	1.34 ± 0.04*†	0.85 ± 0.04*	0.75 ± 0.03*
Carbon dioxide pressure (mm Hg)	52 ± 1	51 ± 1	52 ± 3	48 ± 2	51 ± 2	53 ± 2
pH	7.40 ± 0.01	7.40 ± 0.01	7.33 ± 0.03	7.35 ± 0.03	7.28 ± 0.08*	7.19 ± 0.03*†
Heart rate (beats/min)	187 ± 5	180 ± 6	174 ± 10	166 ± 12	154 ± 12‡	161 ± 12
Blood pressure (mm Hg)	58 ± 6	59 ± 5	57 ± 4	64 ± 3	73 ± 8	56 ± 4
Central venous oxygen ($ml \cdot kg^{-1} \cdot min^{-1}$)	402 ± 37	460 ± 50	418 ± 47	447 ± 66	261 ± 46*	268 ± 39*

Mean ± SEM; $n = 10$.
*$p = 0.01$, normoxia vs hypoxia.
†$p = 0.01$, hypoxia alone vs hypoxia with meclofenamate.
‡$p = 0.05$, normoxia vs hypoxia.
§$p = 0.01$, hypoxia alone vs hypoxia with meclofenamate.

oxygen-poor gas mixture caused fetal Po_2, pH, and base excess to fall, whereas Pco_2 did not show any significant change during hypoxia both with and without meclofenamate pretreatment (Table III). These results suggest that our experimental approach allowed us to expose the fetus to a selective hypoxic insult. There were two degrees of hypoxia that were comparable in all animals, a milder one associated with oxygen content between 1 and 1.5 mmol/L and a more severe one associated with oxygen content <1 mmol/L.

Hypoxia did not induce significant changes in fetal arterial pressures, whereas fetal heart rate showed a statistically insignificant decline with mild hypoxia and a significant decline of 18% with severe hypoxia (Table III).

The effect of mild and severe hypoxia on combined cardiac output and renal blood flow are shown in Table III and in Fig. 1. In Fig. 2, blood flows are expressed as percentage of control to account for the marked interanimal variability. Severe hypoxia caused a significant fall in both combined ventricular output and renal blood flow.

Fetal response to hypoxemia during meclofenamate infusion. As seen in Table III, prolonged pretreatment with meclofenamate did not alter the fetal blood pressure or combined ventricular output response to moderate and severe hypoxia when compared with hypoxia alone. Whereas pretreatment with meclofenamate decreased renal blood flow during moderate and severe hypoxia as compared with hypoxia alone, the differ-

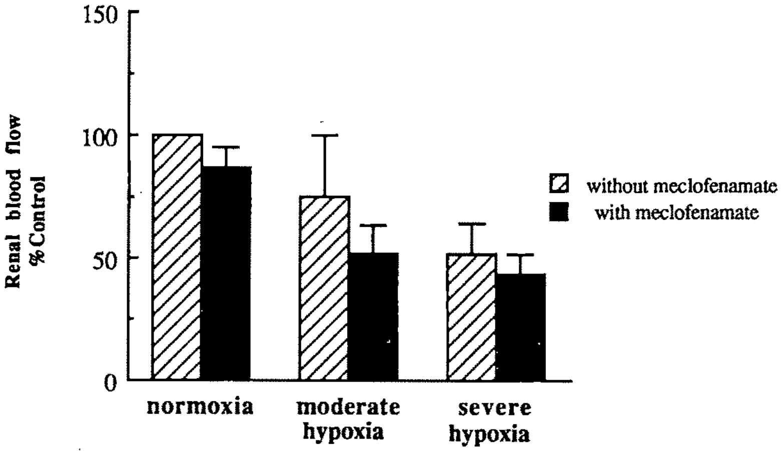

Fig. 2. To minimize interanimal variability each animal was used as its own control. The renal blood flow expressed as percentage of control shows that meclofenamate treatment did not cause a significant change in blood flow as compared to hypoxia alone. Mean ± SEM.

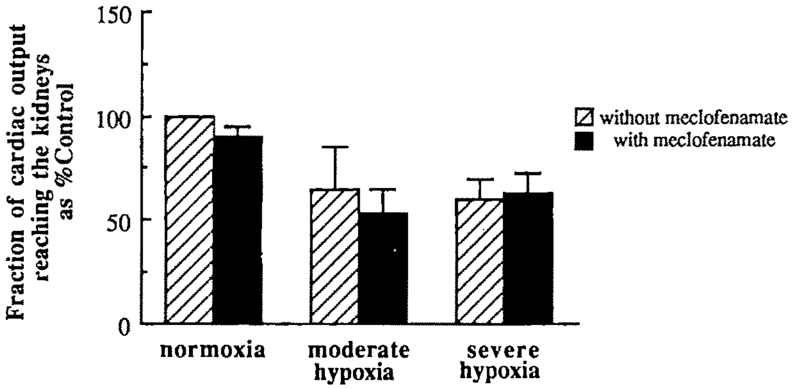

Fig. 3. When renal blood flow was expressed as the fraction of cardiac output perfusing the kidney, meclofenamate treatment did not cause a significant change in this measure as compared with hypoxia alone. Mean ± SEM.

ences did not reach statistical significance, as seen in Fig. 1. To decrease the effect of interanimal variability the results in Fig. 2 were expressed as percentage of control and in Fig. 3 renal blood flow is expressed as percentage of combined ventricular output. Again, no statistically significant effect of meclofenamate pretreatment on the response of renal blood flow to moderate and severe hypoxia could be demonstrated.

Comment

The results of this study demonstrate that prolonged treatment with meclofenamate does not significantly alter basal renal blood flow in fetal sheep. These data are in accordance with those of Rudolph et al. that showed no change in renal blood flow when either prostaglandins or inhibitors of their synthesis are administered to fetal lambs.[14, 15] Apparent discrepancies between our data and those of earlier studies may be a result of study design. Millard et al.[9] showed a reduction in renal blood flow after administration of their indomethacin or meclofenamate. Thirty minutes after the intravenous injection of either indomethacin or meclofenamate renal blood flow, as seen in the electromagnetic flow recordings, had returned to control levels, suggesting that the effect on renal blood flow was transient. To avoid studying such transient and short-lived responses, we chose to study the effect of meclofenamate on renal blood flow after a continuous 4-hour infusion of the drug.

Fetal breathing provides a sensitive and unique measure of prostaglandin activity. We therefore maximized the peripheral action of meclofenamate by choosing an administration protocol known to cause both maximal fetal ventilatory activation and supression of prostaglandin E_2 serum cocentration. In particular, Hohimer et al. have demonstrated that meclofenamate starts to cause its maximal effect on fetal ventilation after 3 to 4 hours of infusion.[16] The dosage of meclofenamate that we used was the same as that used in previous studies and known to inhibit prostaglandin synthesis in the rabbit heart[17] and to decrease plasma prostaglandin E_2 in fetal sheep.[18]

In our experiments, severe hypoxia caused a significant decrease of renal blood flow. Meclofenamate pretreatment did not significantly further the decrease in renal blood flow seen with hypoxia alone. Whereas it is possible that with a larger number of animals the effects of meclofenamate on renal blood flow could become statistically significant, in no case did we see a fall in blood flow >20%. Our data support the conclusion that steady state prostaglandin synthesis inhibition with meclofenamate does not significantly modify the renal blood flow response to moderate or severe hypoxia.

We conclude that in the chronically catheterized fetal sheep prolonged meclofenamate infusion does not significantly alter basal or hypoxic renal blood flow.

We suggest that the oligohydramnios seen in human pregnancies could be better explained by an endocrine rather than a renovascular effect of prostaglandin synthesis inhibitors. In particular, prostaglandins are thought to play an important role in the mediation of renin release, augmentation of the angiotensin II–stimulated aldosterone release from the adrenal gland, and promotion of a state of relative renal insensitivity to the antidiuretic effect of circulating arginine vasopressin.[19, 20] Prostaglandin synthesis inhibitors could increase free water reabsorption by augmenting the antidiuretic action of arginine vasopressin.[19] Clinical observations suggest that the severity of oligohydramnios is unrelated to the length of treatment, is not associated with evidence of intrauterine hypoxia, and is easily and quickly reversible.[5] These observations, like our data, are more consistent with an endocrine mechanism rather than a cardiovascular one for the occurrence of oligohydramnios.

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Isolation and purification of microvascular endothelium from human decidual tissue in the late phase of pregancy

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Normal pregnancy demands significant structural and physiologic adaptations of the uterine microvasculature, to facilitate adequate placentation. In pregnancies complicated by pregnancy-associated hypertension (preeclampsia), this process is defective, resulting in a high incidence of intrauterine growth retardation. The microvascular endothelium, a focal point for both initiation and inhibition of coagulation and control of vascular tone, may well contribute to development and aggravation of these abnormalities. We describe a method for isolation, purification, and culture of human decidual endothelial cells from biopsies performed at the time of cesarean section. The separation procedure is technically simple, requires only a small piece of tissue, and takes approximately 2 hours to perform. Some of the unique features of these cells in culture are outlined. This technique will permit the close examination of various aspects of the function of these cells in normal pregnancy, and their comparison with cells from pregnancies complicated by hypertension. (AM J OBSTET GYNECOL 1991;165:191-6.)

Key words: Decidua, endothelium, cell culture, human pregnancy

Pregnancy-associated hypertension (preeclampsia) is a common problem, associated with considerable maternal and perinatal morbidity, and characterized by dysfunction in many organs, most if not all of the clinical abnormalities explicable on the basis of disordered coagulation and vasoconstriction in many vascular beds, with resultant diminution in perfusion. In this condition, many changes have been described in both maternal intrauterine and placental vessels, although sampling probems have been encountered by many authors in the study of the placental bed.[1] It is generally agreed that there is relative failure of the expected migration of trophoblastic cells and alteration of maternal endothelial and musculoelastic tissue.[2] In addition, the uterine decidual and basal vessels display fibrin deposition, intramural accumulation of fat-laden macrophages, and a perivascular mononuclear cell infiltrate.[3] The initiating events for these abnormalities have never been fully elucidated. According to both Fox[4] and Nesbitt,[5] reduced uteroplacental blood flow[6, 7] is sufficient to cause virtually all the abnormalites seen in the placenta in preeclampsia, including the excess frequency of thrombi, fibrin deposition, and infarction in the placental circulation.[8] Ultrastructural endothelial cell damage has been described in decidual tissue both adjacent to and distant from the placental bed site.[1]

A disturbance in endothelial cell function could well contribute to the pathogenesis of the clinical syndrome of preeclampsia.[9] The vascular endothelium can provide a focal point for the initiation of processes inhibiting and promoting activation of coagulation.[10] It is also recognized as an important functional unit involved in the regulation of smooth muscle tone,[11] and is capable of producing both vasodilator and vasoconstrictor substances. Microvascular endothelium derived from different organs displays distinctive tissue-specific characteristics.[12, 13] As the most significant physiologic adaptations in the maternal microvasculature (and their abnormalites) are seen at the maternofetal interface, examination in vitro of endothelial cell function from this site is important.

An essential first step in the examination of decidual endothelial cell function is their isolation and culture. This article describes a method to isolate decidual endothelial cells with the use of dynabeads coated with the lectin UEA 1, which binds specifically to human endothelial cells by means of a cell surface glycoprotein that contains L-fucose residues.[14] Preliminary results of the growth characteristics of isolated decidual endothelial cells are also presented.

Material and methods

Subjects. The subjects were normotensive, multiparous women undergoing elective repeat cesarean section delivery for obstetric indications. The study re-

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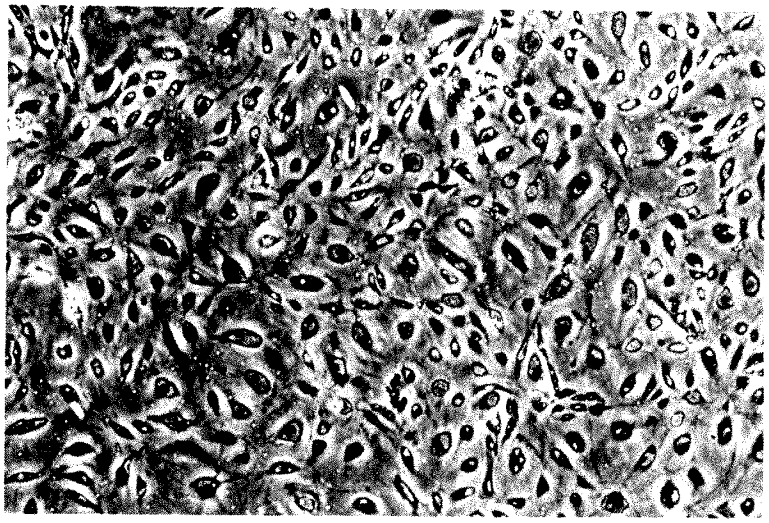

Fig. 1. Decidual endothelial cells at confluence (day 8, primary culture). The cells form a cobblestone-like monolayer. UEA 1–coated dynabeads can be seen attached to some of the cells (original magnification × 160).

ceived prior approval from the Medical Research Ethics Committee of the Royal North Shore Hospital and Northern Sydney Area Health Service. For this preliminary study, informed verbal consent was obtained from subjects before delivery of infants.

Isolation and culture of decidual endothelium. A shallow decidual biopsy specimen was taken by the obstetrician after delivery of the placenta and was immediately placed in cold calcium- and magnesium-free Hanks' buffered salt solution (Cytosystems, Sydney, Australia). Endothelial cells were isolated as described in detail previously for the isolation of synovial endothelium.[15] Sterile procedures were used throughout. Briefly, the specimen was cut into 5 mm cubes and incubated at 37° C for 7 to 8 minutes in trypsin 1:250 (0.12 gm/dl; ICN Biomedicals, Cleveland) and Pronase (0.08 gm/dl; Calbiochem, San Diego). The resultant fragments were washed five times with Hanks' balanced salt solution, capillary segments were squeezed from the cut edges by downward pressure with the flat edge of a scalpel blade and the resultant mixed cell population was collected, centrifuged (1000 *g*, 5 minutes) and resuspended in 80 μl of Hanks' balanced salt solution plus 5% fetal calf serum. This cell suspension was incubated at 4° C for 10 minutes with 20 μl of the lectin ulex europaeus I (UEA 1)–coated dynabeads (4 × 10^8 beads/ml), as described previously,[15] and then washed five times with 2 ml Hanks' balanced salt solution plus 5% fetal calf serum, separation after each wash being achieved in a magnetic particle concentrator. In some subjects, the cell-bead complex was dissociated by competitive binding of L-fucose before plating down.

The contaminating cells contained in the wash medium and the cells attached to the beads were resuspended separately in growth medium and plated down on a gelatin-coated 35 mm tissue culture dish (Lux, Flow Laboratories). Cells were routinely maintained in a humidified incubator at 37° C in a 95% air, 5% carbon dioxide atmosphere. The growth medium used was Wissler's:McCoy's (1:1) medium containing growth factors 0.1 mg/ml endothelial cell growth supplement (Collaborative Research, Bedford, Mass.), 0.1 mg/ml heparin (Sigma Chemical, St. Louis), 2.5 μg/ml fungizone, 100 mU/ml penicillin, and 100 μg/ml streptomycin, with 40% serum: fetal calf serum, pooled human umbilical cord serum, pooled human serum from nonpregnant donors, or pooled serum collected from women in the second trimester of pregnancy (human pregnancy serum).

Cells were examined by phase contrast microscopy, refed at 2-day intervals, and passaged at confluence with the use of trypsin 1:250 (Difco, Detroit) 0.05%/0.02% (weight/volume) ethylenediaminetetraacetic acid in phosphate-buffered saline solution. Endothelium was identified by its expression of von Willebrand factor antigen with immunofluorescence.[16, 17]

Other cell lines. Human umbilical vein endothelial cells obtained from fresh umbilical cords by the method of Jaffe et al.[18] and used as a control cell population for decidual endothelial cell replication experiments, were a gift from Mr. T. To, Sutton Laboratory, Royal North Shore Hospital.

Cell replication. Cell replication experiments were conducted in triplicate by the method of Oliver et al.[19] Cells (passage 4) were plated into 96-welled plates (0.6 to 1.0 × 10^4 cells/well) in Wissler's:McCoy's medium (1:1) with endothelial cell growth supplement, heparin,

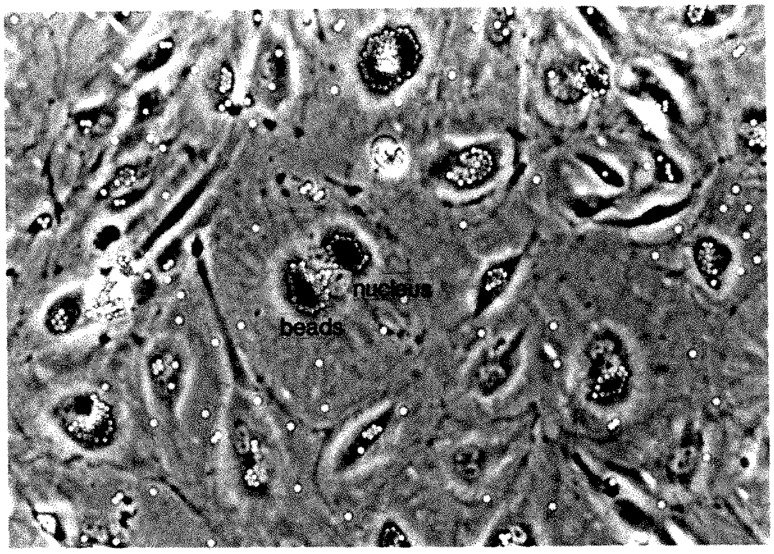

Fig. 2. UEA I–coated dynabeads bound to decidual endothelial cells in culture. A dividing cell is marked, showing the two nuclei free of beads, whereas the cytoplasm is densely covered by them. (Day 3 of primary culture, original magnification ×260).

and 10% to 40% fetal calf serum or human pregnancy serum. The effects of 40% serum without additional growth factors, and of estradiol (Steraloids, Wilton, N.H.) (at final concentrations of 50 and 500 ng/ml) were also assessed, as was the effect of doubling the media concentration of endothelial cell growth supplement in the presence of 40% fetal calf serum. After 2 or 4 days' incubation in these media, cells were washed twice with 0.9% sodium chloride, fixed in 10% formol saline solution, and stained with methylene blue (1.0 gm/dl). The plates were washed four times with 0.01 mol/L borate buffer (pH 8.5), the dye was then eluted with 1 : 1 (vol/vol) ethanol/0.1 mol/L hydrochloric acid and the optical density (OD) was read at 620 nm in a plate reader.

Results

Endothelial cells with >99% purity were obtained from 11 of 13 biopsy specimens. Within 24 hours of isolation, cells with beads bound to them had typical endothelial morphology and had formed discrete rounded colonies. By 8 days of primary culture, they formed a typical confluent cobblestone-like monolayer (Fig. 1) with the beads still attached, although the number per cell was decreased. In no instance was there overgrowth by contaminating cells. Cells that had previously been dissociated from the beads with L-fucose were indistinguishable in morphology and growth pattern in culture from those that had not been subjected to this procedure (data not shown).

In addition to their typical morphology, these cells were identified as endothelial by: (1) their expression of receptors that bind UEA I (Fig. 2); and (2) their expression of von Willebrand factor antigen, as measured by indirect immunofluorescence (Fig. 3). By light microscopy, UEA I–coated beads were bound to all cells initially isolated as endothelial, the number varying between 1 and 80, with a median of nine beads per cell. Binding to the cell membrane did not occur directly over the nucleus, a feature noted previously with human umbilical vein endothelial and synovial endothelial cells.[15] Those cells collected in the wash that had not bound to the UEA I–coated beads were a mixed population, predominantly comprising fibroblast-like cells.

Cell proliferation

General. In initial experiments, decidual endothelial cells plated down in growth medium containing 40% fetal calf serum, 40% human cord serum, or 40% pooled human serum from nonpregnant donors grew very slowly. Those whose culture medium contained 40% pooled serum collected from normal women in the second trimester of pregnancy had the most rapid growth and replication rate. With this medium, cells have been successfully grown for up to 7 passages.*[1] After an inital period of rapid growth, subculture has been possible every 18 to 22 days. During this time, the morphology of the cells has remained unchanged.

Effect of serum on cell replication. The effect of a range of concentrations (10% to 40%) of fetal calf serum or human pregnancy serum on the replication rate of decidual endothelial cells was examined at passage 4. In Fig. 4, results are shown for day 2 of a typical replication experiment. As expected, no significant change in

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*At the time of preparation of this manuscript.

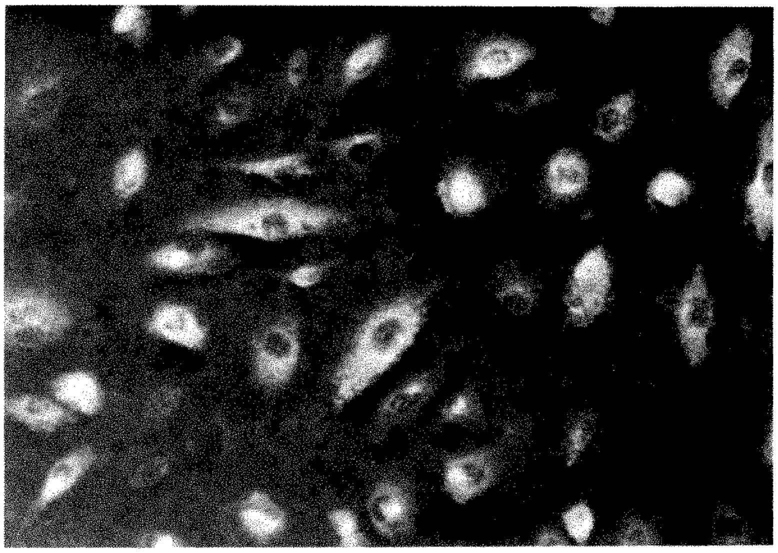

Fig. 3. Positive immunofluorescence of decidual endothelium for von Willebrand factor antigen (original magnification ×510).

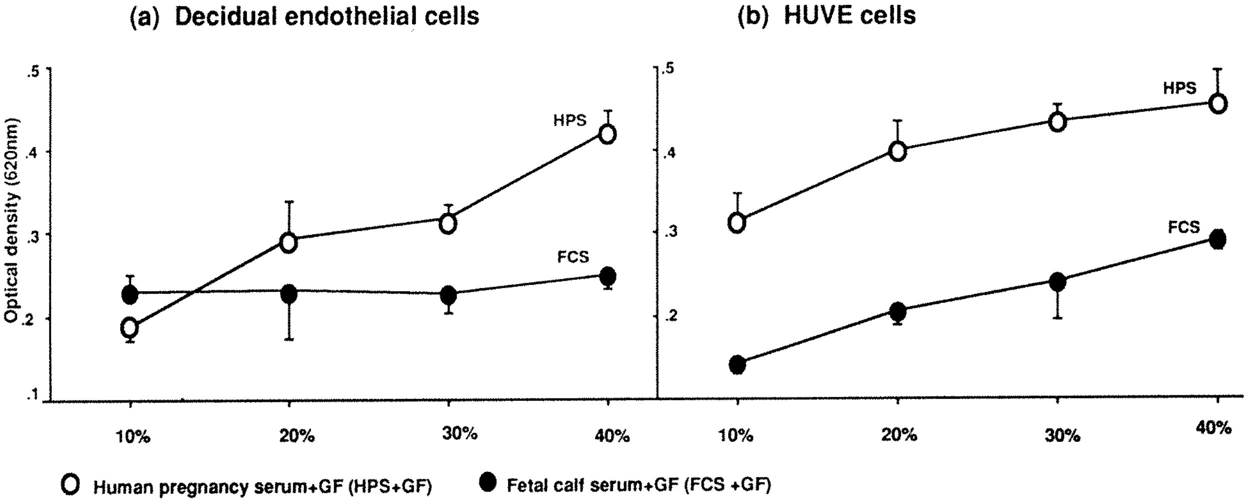

Fig. 4. Comparative rates of growth of decidual and umbilical vein endothelial cells. Day 2 results (mean ± SD) are given for a representative experiment.

growth rate was observed with the addition of fetal calf serum to the growth medium over this range. By contrast, the addition of human pregnancy serum (Fig. 4, *a*) was associated with a concentration-dependent increase in cell replication.

Human umbilical vein endothelial cells at the same passage responded differently from decidual endothelial cells (Fig. 4, *b*). An increase in the concentration of fetal calf serum from 10% to 40% resulted in an increase in the replication rate (OD 0.142 U vs OD 0.295). There was a similar concentration-dependent increase in growth rate in response to human pregnancy serum, but from a substantially higher baseline value (OD 0.323), indicating greater augmentation of growth, even at human pregnancy serum concentrations as low as 10%.

Human pregnancy serum stimulated the growth of large-vessel human umbilical vein endothelial cells to a greater extent than that of decidual cells. This difference was most marked in wells with the lowest concentration of human pregnancy serum (10%) (decidual cells mean OD 0.183 vs. human umbilical vein endothelial cells OD 0.323, $p < 0.02$). Whereas the trend was similar in the presence of higher concentrations of human pregnancy serum, the difference was less marked in the presence of 40% serum (mean OD 0.418 vs. 0.465) and failed to reach statistical significance after 2 days. However, at day 4 there was a more clear-cut

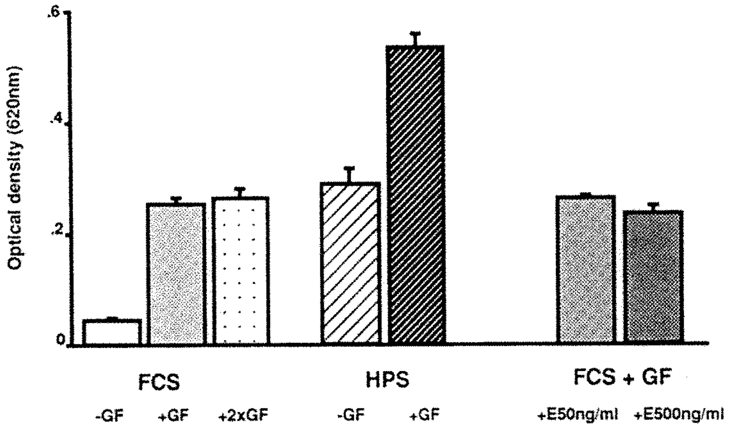

Fig. 5. Factors influencing decidual endothelial cell growth rate. Day 4 results (mean ± SD) are given for a representative experiment. (*HPS*, Human pregnancy serum; *FCS*, fetal calf serum; *E*, estradiol; *GF*, growth factors; *2 × GF*, double endothelial cell growth supplement dose).

difference between the two cell populations at this concentration of human pregnancy serum (decidual cells OD 0.527 ± 0.029 vs. human umbilical vein endothelial cells OD 0.900 ± 0.028, $p < 0.01$).

Nature of growth factor in human pregnancy serum. To examine the nature of the growth factor in human pregnancy serum a little further, we compared its effect on decidual endothelial cells (passage 4) with the growth factors endothelial cell growth supplement and heparin. Results are shown in Fig. 5. Decidual cells incubated in fetal calf serum without growth factors failed to replicate, whereas the addition of growth factors augmented levels to those seen with human pregnancy serum alone. Doubling the dose of growth factors did not stimulate more cell growth. Human pregnancy serum combined with growth factors, however, resulted in a higher cell replication rate than either stimulus alone.

Estrogen, secreted in greatly increased amounts in normal pregnancy, is a significant trophic hormone, and is one candidate for the role of the additional growth factor in human pregnancy serum. The effect of supplementation of the fetal calf serum medium with growth factors by estrogen is shown in Fig. 5. Estradiol, the major estrogen metabolite circulating in pregnancy, was added at two concentrations, 50 ng/ml (within the range of normal plasma estradiol in pregnancy) and 500 ng/ml. As shown in Fig. 5, neither of these concentrations of estradiol was associated with an increase in replication rate.

Comment

The primary goal of the experiments described here was to isolate and culture endothelial cells from late human pregnancy decidual tissue. This has been achieved: a pure population of viable cells that is von Willebrand factor-positive has been obtained with the morphologic features and growth pattern typical of endothelial cells.[16, 17] These cells have been maintained in culture for prolonged periods of time, and retain typical endothelial morphology.

Endothelial cells from different tissue sources are known to have different phenotypic characteristics.[12, 13] Our experiments have shown that decidual microvascular endothelial cells have different growth requirements from a control large vessel endothelial cell population (human umbilical vein endothelial cells). They grow more slowly. This finding has been noted for other microvascular beds.[20] They also have different nutrient requirements. A previous brief report[21] mentions an effect of human pregnancy serum in reducing the requirement of skin microvascular endothelial cells for a high concentration of human serum, suggesting the presence of a powerful growth factor. Our results confirm and extend that observation. A number of factors are known to stimulate endothleial cell growth. We have shown that the effect of human pregnancy serum is additive and could be separate from that of endothelial cell growth supplement and heparin, inasmuch as doubling the concentrations of these two growth factors produced no further augmentation of growth in fetal calf serum–containing culture medium.

The nature of the growth stimulating factors in human pregnancy serum is unexplained. Addition of estradiol (in concentrations at or above those seen in normal pregnancy) to fetal calf serum with growth factor did not reproduce the effect. It is still possible that estrogen could produce such an effect in vivo, either metabolized to a more active form or acting as a cofactor. There are, of course, many other hormones, cytokines, and biologic response modifiers secreted in normal pregnancy that could produce such an effect alone or in combination. Experiments with antisera to these would eludicate this. Future work will address this question.

Although the decidual endothelial cells isolated with this technique are normal in apperance, in viability (as assessed by trypan blue exclusion), and in growth pattern, it is possible that the harvesting and purification procedures may have induced subtle alterations in endothelial cell function, such as cytokine production. These questions are being examined.

Because an early and central feature of preeclampsia is the abnormal interaction between placental tissue and decidual endothelium, it is clearly important to study tissue from this source. Whereas this article evaluates decidual endothelium isolated from normal pregnant subjects, we are currently applying this technique to its isolation from hypertensive women, and will compare the behavior of such cells to those of normotensive women.

We thank our obstetric colleagues for collection of biopsy material and B. Nissen and K. Jenkins for expert technical assistance.

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SYMPOSIUM

Pathogenesis of Endometriosis and Adenomyosis

Guest Editors
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Introductory remarks

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Despite recent advances in the diagnosis and treatment of endometriosis, long-standing clinical problems with the disease still persist. For instance, patients with mild endometriosis may have intractable pain, whereas patients with severe disease are sometimes free of symptoms. As for infertility, the cause of infertility in women with severe disease is intuitively understandable. However, the cause of infertility in women with minimal or mild disease is obscure, thus challenging a direct cause-and-effect relationship. The natural history of the disease is still poorly understood. It is unclear whether the reappearance of endometriosis after complete surgical removal results from the remaining microscopic foci or is a consequence of newly developed lesions, because circumstances predisposing to the development of the disease, such as menstrual reflux, coelomic metaplasia, and a favorable hormonal environment, may be unaltered after treatment.

To resolve these issues, much attention should be directed to understand the etiology and histogenesis of endometriosis. At present several theories have been postulated to explain the histogenesis of endometriosis, including the implantation theory, benign metastatic theory, mechanical transplantation theory, coelomic metaplasia theory, müllerian cell rests theory, and Wölffian cell rests theory. However, no single theory can provide a proper explanation for the clinical presentation of all cases of endometriosis. Therefore this conference addressed updated concepts about the etiology and histogenesis of endometriosis; investigators who are actively working in the field of endometriosis gathered together to discuss the histogenesis and pathophysiology of endometriosis based on experimental models, histopathologic studies, and clinical findings.

The conference participants also dealt with adenomyosis. Although adenomyosis is considered a different clinical entity, a deeper insight about the etiology and histogenesis of this disease may prove useful in understanding the etiology and histogenesis of endometriosis.

Finally, we express our gratitude and appreciation to Professor Masahiko Mizuno, Chairman of the Department of Obstetrics and Gynecology, University of Tokyo, for his kind and always excellent advice and constant encouragement. We also acknowledge the educational grants from Tokyo Tanabe Co., Ltd., which made this conference and these proceedings possible.

6/0/29993

Subserosal adenomyosis: A possible variant of pelvic endometriosis

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A total of 128 patients with adenomyosis were examined clinically and histologically to investigate the pathogenesis of subserosal adenomyosis. In this study 15 (11.7%) cases were classified as subserosal adenomyosis on the basis of localization of heterotopic endometrial tissues that consisted of glands and stroma. These were subclinical and incidental. The uterus was not enlarged in this type of adenomyosis. Distribution of the ectopic tissues and the patients' mean age at the time of the hysterectomy suggest that subserosal adenomyosis may develop as a variant of pelvic endometriosis. (AM J OBSTET GYNECOL 1991;165:198-201.)

Key words: Adenomyosis, subserosal adenomyosis, pathogenesis, pelvic endometriosis

Typical adenomyosis consists of scattered fragmented lesions of endometriotic tissue, including both glands and stroma, in the myometrium of the enlarged uterus.[1] The distribution and number of heterotopic endometrial foci vary from patient to patient. Some have only a few foci in the uterus, which are located mainly adjacent to the serosa. An analysis of these patients with an unusual distribution of heterotopic endometrial tissue may help elucidate the pathogenesis of adenomyosis.

In this study patients with adenomyosis were examined from a clinical and histopathologic standpoint. Special emphasis was placed on patients with an uncommon distribution of heterotopic endometrial tissue to investigate the mechanisms involved in the pathogenesis of subserosal adenomyosis.

Case and methods

Specimens from 128 patients with histologically confirmed adenomyosis were examined retrospectively. All had undergone hysterectomy at Kyoundo Hospital in Tokyo between January 1989 and July 1990. In one third of these patients, unilateral or bilateral salpingo-oophorectomy had been performed simultaneously. The ages of the patients at the time of the operation and each clinical diagnosis were taken from the clinical records.

The cut surface of the posterior wall of the uterus was examined histologically in all cases. The rest of the uterus was cut in a stepwise manner and then inspected macroscopically. When necessary additional blocks were used for histologic examination. All preparations were stained with hematoxylin and eosin. Distribution of heterotopic endometrial foci and the histologic findings in them were described. On the basis of results of histologic examination, all patients were divided into two groups: those with subserosal adenomyosis and the remainder, which were subdivided according to the topographic location of the foci in the uterus. The site of the lesion, whether corpus or cervix, was also recorded.

Results

Of the 128 cases of adenomyosis, 15 (11.7%) corresponded to the subserosal type. The foci were few in number and were located close to the subserosal surface. In some instances, a contiguous but shallow extension of the subserosal foci was found in the myometrium adjacent to the serosa. The mean age ± standard deviation of these patients was 42.9 ± 4.6 years. The mean age ± standard deviation of the patients with typical adenomyosis was 45.8 ± 7.0 years.

Both the clinical diagnosis and the postoperative histologic findings in the patients with subserosal adenomyosis are shown in Table I.

Clinically subserosal adenomyosis was not detected in any of the patients. All of them had undergone hysterectomy for diseases other than adenomyosis. Concomitant ovarian endometriosis was also found in six of the patients with subserosal adenomyosis; their ages ranged between 35 and 42 years.

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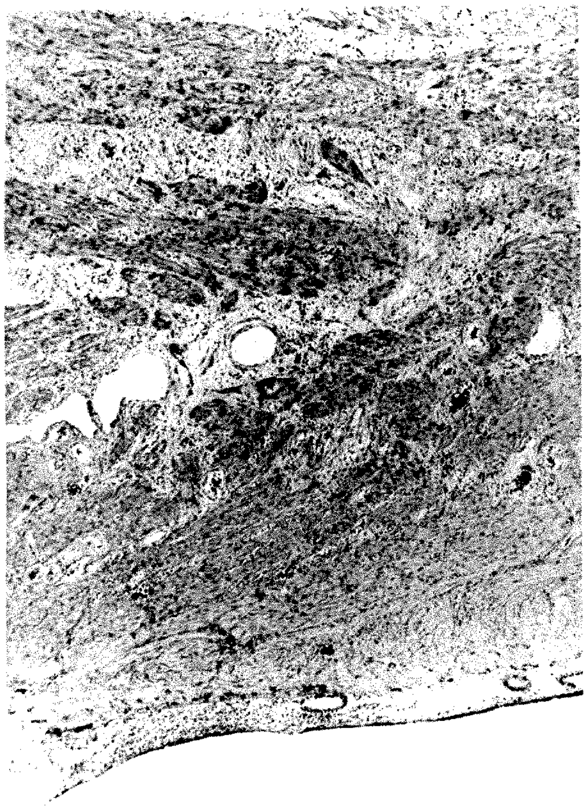

Fig. 1. Subserosal adenomyosis (case 7). Ectopic endometrial tissue including glands and stroma was located in subserosal area. (Hematoxylin and eosin stain; original magnification ×40.)

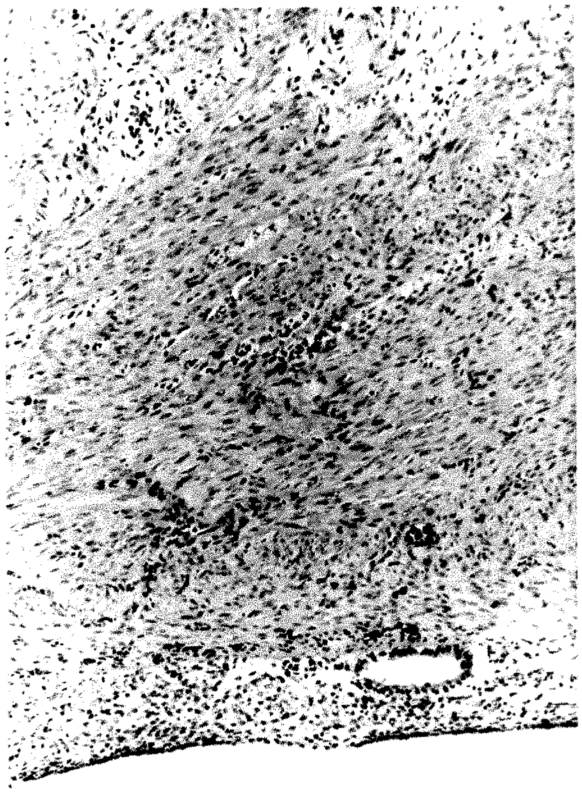

Fig. 2. High-power view of Fig. 1. Heterotopic tissue was found between serosa and myometrium. (Hematoxylin and eosin stain; original magnification ×100.)

Table I. Clinical diagnosis and histologic findings in patients with subserosal adenomyosis

Case No.	*Age (yr)*	*Clinical diagnosis*	*Main histologic findings*	*Site of ectopic tissue*
1	35	Myoma uteri + endometriosis	Leiomyomas of uterus and ovarian endometriosis	Corpus; posterior, lower
2	35	Endometriosis	Ovarian endometriosis	Corpus; posterior, lower
3	39	Myoma uteri + endometriosis	Leiomyomas of uterus and ovarian endometriosis	Corpus; posterior, middle
4	40	Myoma uteri + endometriosis	Leiomyomas of uterus and ovarian endometriosis	Corpus; posterior, middle
5	40	Myoma uteri	Myomatous hypertrophy of uterus	Corpus; posterior, middle
6	41	Myoma uteri + endometriosis	Leiomyomas of uterus and ovarian endometriosis	Corpus; posterior, middle
7	42	Myoma uteri	Myomatous hypertrophy of uterus	Corpus; posterior, lower
8	42	Myoma uteri	Myomatous hypertrophy of uterus	Corpus; posterior, middle
9	42	Myoma uteri + endometriosis	Ovarian endometriosis	Corpus; posterior, middle
10	46	Myoma uteri	Leiomyomas of uterus	Cervix; posterior
11	47	Myoma uteri	Leiomyomas of uterus	Corpus; anterior, upper
12	48	Ovarian cancer	Clear cell adenocarcinoma of ovary	Corpus; posterior, upper
13	48	Myoma uteri	Leiomyomas of uterus	Cervix; posterior
14	48	Myoma uteri	Leiomyomas of uterus	Cervix; posterior
15	50	Myoma uteri	Leiomyomas of uterus	Corpus; anterior, middle

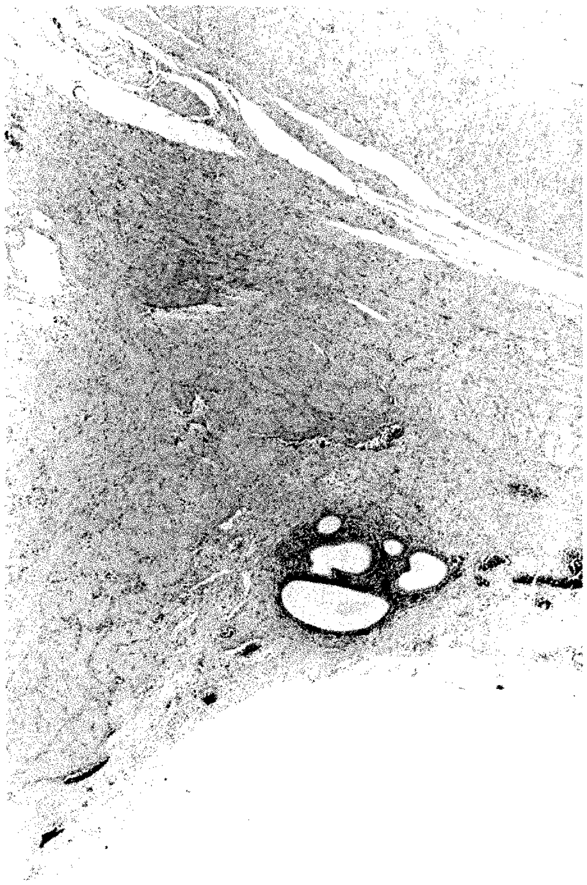

Fig. 3. Subserosal adenomyosis (case 6). Ectopic tissue was scattered in myometrium just below subserosa. Leiomyoma was also found *(upper right side)*. (Hematoxylin and eosin stain; original magnification ×40.)

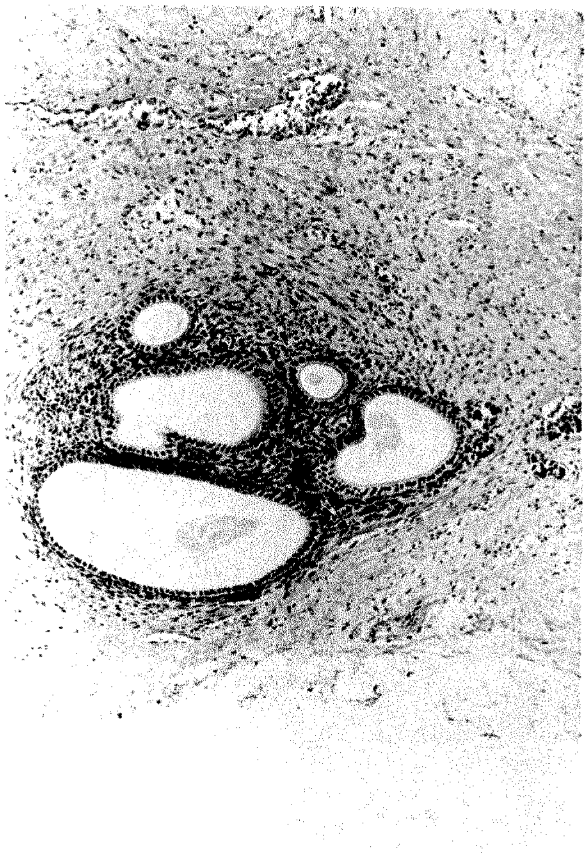

Fig. 4. High-power view of Fig. 2. Histologic findings of heterotopic tissue resemble those of basal layer of eutopic endometrium. (Hematoxylin and eosin stain; original magnification ×100.)

Regarding the location of ectopic endometriotic tissue, three were in the cervix and the remainder were in the uterine body.

The histologic findings in the ectopic endometrial tissue in subserosal adenomyosis were similar to those of the basal layer of the eutopic endometrium (Figs. 1 to 4). All foci were composed of glandular elements surrounded by stromal tissue. No hemorrhagic changes were identified in the heterotopic glands or in the stroma. There were no close correlations between the ectopic endometrial tissue and the vessels. Neither atypical nor malignant cells were found in these ectopic tissues.

Comment

The pathogenesis of adenomyosis is not well understood. In most cases of adenomyosis, ectopic endometrial tissue is found scattered within the myometrium in close correlation with the eutopic endometrium. Elongation of the endometrial basal layer into the myometrium is thought to be one of the possible mechanisms involved in the pathogenesis of this condition. However, in subserosal adenomyosis ectopic endometrial tissue is distributed in an area distant from the eutopic endometrium. It seems impossible to state that this type of disease occurs as a result of direct proliferation of the eutopic endometrium. Migration of the tissue via lymphatic channels, as postulated by Javert,[2] should also be considered as a possible mechanism. However, lymphatic permeation could not be confirmed in the cases reported here.

Previously it was suggested that subserosal adenomyosis may be a variant of pelvic endometriosis.[3-5] Some evidence, including the distribution of ectopic endometrial tissue supports this hypothesis. The mean age of the patients with subserosal adenomyosis reported in this study was 3 years less than that of the rest of the patients with adenomyosis; that is, they were about the same age at which pelvic endometriosis usually develops (the fourth decade on average).[1] It is believed that adenomyosis is not commonly associated with endometriosis, even in patients with widespread endometriosis. However, the high incidence of patients with concomitant subserosal adenomyosis and endometriosis found in this study indicates a close correlation between these two conditions.

Thus subserosal adenomyosis can be regarded as a possible variant of pelvic endometriosis, although further studies are required to confirm this theory.[6]

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Histologic study of endometriosis and examination of lymphatic drainage in and from the uterus

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Histologic study of endometriosis and investigation of lymphatic drainage in and out of the uterus were conducted to obtain more information on the histogenesis of endometriosis. Endometriosis is a disease originating from the normal endometrium, specifically from its basal layer. Internal endometriosis (adenomyosis) is caused mainly by direct invasion of the endometrium into the spaces located in the connective tissue of the muscle layer. Serous and ovarian types of endometriosis are frequently found together with adenomyosis. Endometrial fragments in vessels are detected in 4.5% of cases of endometriosis. Observation of serial sections proved that serous endometriosis is caused mainly by continuous or semicontinuous invasion of the tunica muscularis spaces primarily from adenomyosis lesions and partly from the endometrium. Confirmation of lymph flow into the ovary from the uterine body strongly suggests that ovarian endometriosis arises as a consequence of vascular (lymphatic) transport of endometrial fragments from adenomyosis lesions, serous endometriosis lesions, or the endometrium. (AM J OBSTET GYNECOL 1991;165:201-9.)

Key words: Histogenesis, morphology, lymphatic drainage, endometriosis

Endometriosis, which can be pathologically considered a benign proliferative lesion, is classified into internal endometriosis (adenomyosis) and external endometriosis (serous and ovarian endometriosis).

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		morphology					function						
		constructive	electromicroscopic finding		hormonal reaction		E_2, P receptor		tissue E_2, P		variation of nuclear DNA histogram		tumor makers CA 125, CA 199, EMA
		cells	ep. c.	st. c.	ep. c.	st. c.	activity	variation	amount	variation	ep. c.	st. c.	
Endometrium	functional layer	◎	◎	◎	⧻	⧻	⧻	⧻	⧻	⧻	⧻	+	⧺
	basal layer	◎	○	○	+	+	+	+	⧺	⧺	⧺	+	+
Endometriosis	internal	◎○	○	○	⧺~+	⧺~+	+	+	+	+	⧺	+	+
	external	◎○△	○	○	+~−	+~−	+	+~−	+	+~−	⧺~+	+	+

morphological resemblance ◎ high, ○ recognizable, △ low
functional characteristics ⧻ very strong, ⧺ strong, + recognizable weak
ep. c. = epithelial cell, st. c. = stromal cell

Fig. 1. Morphologic and functional characteristics of endometriosis: Comparison with normal endometrium.

Table I. Theories concerning the histogenesis of endometriosis

Theories assuming origin in tissues other than the endometrium
Wolffian duct theory (Von Recklinghausen,[7] 1896)
Müllerian duct theory (Russell,[8] 1899)
Coelomic metaplasia theory (Iwanoff,[3] 1898; Meyer,[4] 1903)
Hormonal stimulation theory (Novak,[9] 1931)
Induction phenomenon theory (Levander and Normann,[5] 1955; Merrill,[6] 1966)
Theories assuming origin in the uterine endometrium
Direct-extension theory (Cullen,[1] 1908)
Implantation theory (Sampson,[2] 1921)
Benign metastasis theory (Halban,[10] 1924)
Uterotubal theory (Phillip and Huber,[11] 1939)
Mechanical transplantation (Greenhill,[12] 1942)

The cause of endometriosis has not yet been determined conclusively, although numerous theories concerning its histogenesis have been proposed. At present the direct-extension theory[1] for internal endometriosis and the implantation theory,[2] the coelomic metaplasia theory,[3, 4] and the induction theory[5, 6] for external endometriosis are the most favored (Table I).

The morphologic and functional characteristics of endometriotic lesions (internal and external) have already been compared with those of the endometrium (functional and basal layer) to determine the histogenesis of endometriosis (Fig. 1), and it was concluded that endometriosis was likely to originate in the endometrium because both internal and external endometriosis lesions resembled the basal layer of the endometrium.[13]

On the basis of these previous findings, in the present study adenomyosis and pelvic endometriosis were investigated from a histopathologic standpoint and lymphatic drainage in and out of the uterus was studied to determine the histogenesis of these conditions.

Material and methods

Histologic examination. To determine the relationship between adenomyosis and serous endometriosis, the incidence of lesions by site and the rate of occurrence of combinations of these lesions were determined in 278 patients with adenomyosis and pelvic endometriosis. In addition, 200 semiserial sections (5 μm thick) taken approximately 45 μm apart, which included the endometrium and the serous membrane, were prepared from 14 patients with serous endometriosis and examined after staining with hematoxylin and eosin. The incidence and patterns of endometrial fragments in the vessels were also observed in 290 patients.

Lymphatic drainage. To examine lymphatic drainage, India ink was injected into the uterine cavity after hysterectomy (13 patients). Uteri were stored for 2 days in a cold dark place and fixed in formalin. India ink was also injected directly into the uterine subserous lymphatic vessels with a 30-gauge needle immediately after hysterectomy (7 patients). Uteri were fixed in the same way. In four volunteers who gave their informed consent, India ink (0.2 to 0.3 ml) was injected into the subserous lymphatic vessels during the operation to observe the drainage in situ. To observe lymphatic drainage in the ovaries, 0.1 ml of India ink was injected under the tunica albuginea with the same needle immediately after oophorectomy (15 patients). Ovary samples were fixed in formalin after standing at room temperature for 1 hour to allow the ink to flow along the vessels. Each sample was examined after staining with hematoxylin and eosin.

Electron microscopic procedure. Uteri taken from five patients with adenomyosis were cut into small pieces and immersion fixed in 1% paraformaldehyde and 1.25% glutaraldehyde in 0.1 mol/L cacodylate buffer, pH 7.35, for 10 hours at room temperature; they were then fixed in 1% osmium tetroxide with 0.01 mol/L cacodylate buffer for 1.5 hours. Specimens were

Table II. Incidence of each type of endometriosis

Classification	*No. of cases (%)*
A alone	57 (20.5)
A + O + S	91 (32.7)
A + O	33 (11.9)
O + S	27 (9.7)
A + S	53 (19.1)
O alone	14 (5.0)
S alone	3 (1.1)
Total	278

A, Adenomyosis; *S*, serous endometriosis; *O*, ovarian endometriosis.

Table III. Subserial section of serous endometriosis (n = 14; no adenomyosis in the muscle layer was found in three cases)

	Continuous		*Noncontinuous*	
	G ± *S*	*S only*	*G* ± *S*	*S only*
None A (no gland cell)		1		
A (lesion confined within muscle layer)	6		2	
A (lesion reached serosa)	2			
	8	1	2	0

A, Adenomyosis; *G*, endometrial gland cell; *S*, endometrial stromal cell.

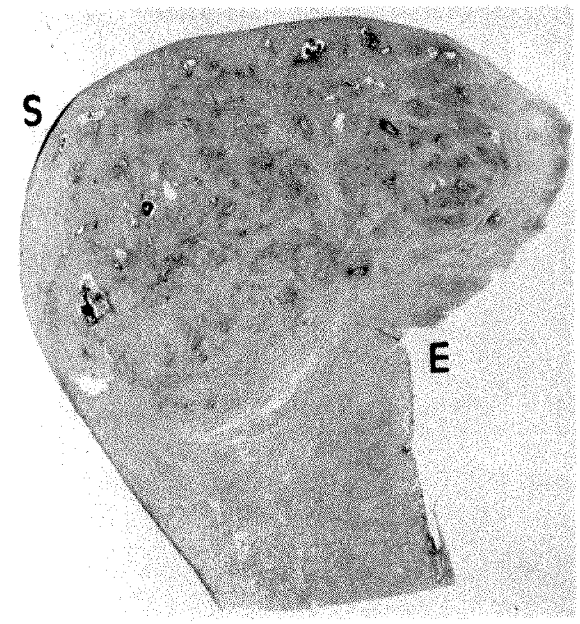

Fig. 2. Adenomyosis associated with serous endometriosis. *E*, Endometrium; *S*, serosa.

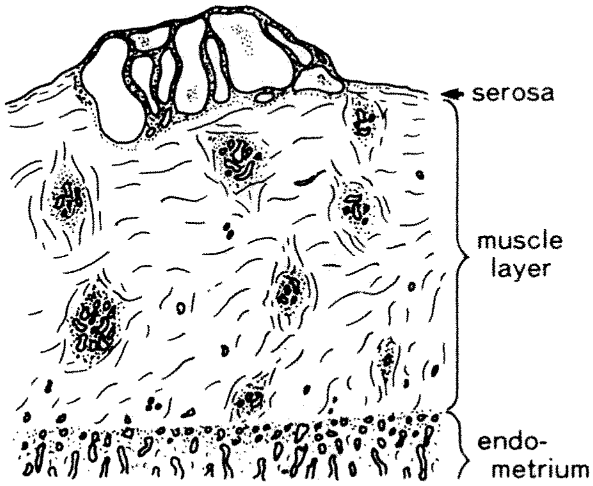

Fig. 3. Adenomyosis and serous endometriosis. Sketch of continuous endometriosis lesion through muscle layer from endometrium to serosa.

dehydrated in graded concentrations of ethanol and embedded in Epon 812. Thin sections (60 to 80 nm thick) were double stained with uranyl acetate and lead citrate and examined with a Hitachi H 300 electron microscope.

Results

Histologic examination. The incidence of endometriosis lesions per site, determined by histologic examination in 276 patients, was 20.5% for adenomyosis alone, 1.1% for serous endometriosis alone, 5.0% for ovarian endometriosis alone, 32.7% for adenomyosis plus serous and ovarian endometriosis, 11.9% for adenomyosis plus ovarian endometriosis, 9.7% for ovarian plus serous endometriosis, and 19.1% for adenomyosis plus serous endometriosis. The incidence of serous endometriosis and adenomyosis combinations and ovarian endometriosis and adenomyosis combinations was high (65.2% and 56.1%, respectively, when adenomyosis alone was excluded from the denominator), whereas the incidences of ovarian endometriosis alone and serous endometriosis alone were low (Table II).

Results of detailed histologic examinations conducted in 14 patients with serous lesions showed a relationship with adenomyosis lesions in 78.5% of these patients (11 of 14) (Table III). Adenomyosis lesions were seen throughout the entire tunica muscularis in two patients (14.3%; Fig. 2). Although these adenomyotic lesions were present in only part of the tunica muscularis in eight patients, continuous invasion into the serous membrane from these adenomyotic lesions was seen in six of eight patients (42.9% of the total examined histologically; Fig. 3), and in two patients (14.3%) the lesions were semicontinuous. In one patient, continuous invasion of stromal cells into the serous membrane through the tunica muscularis was observed.

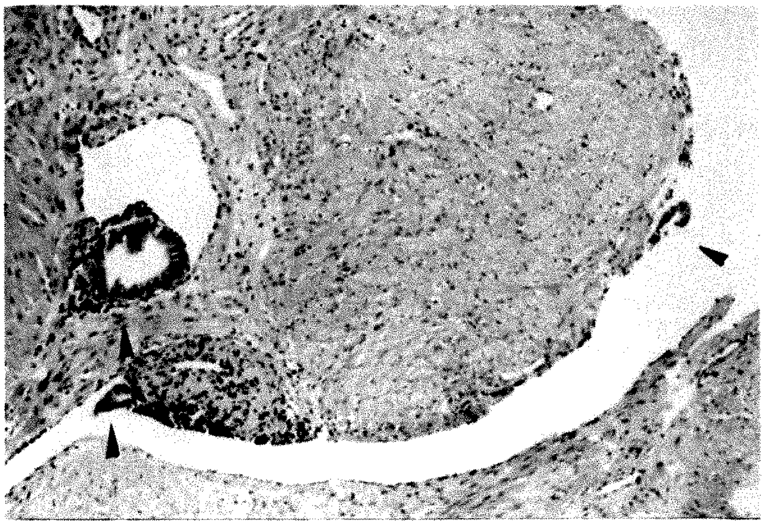

Fig. 4. Endometrial fragments in lymph vessels (attached to wall: *arrow*).

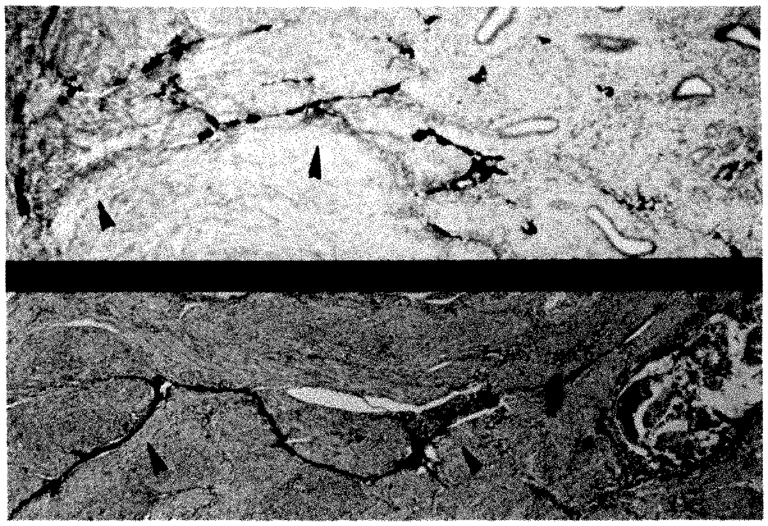

Fig. 5. Demonstration of course of flow (India ink technique). Ink flow is diffused in connective tissue space in superficial layer of endometrium, but in basal layer it is clearly seen in lymph vessel (*top*, adenomyosis case; *bottom*, endometrial cancer). *Arrows* show each lesion.

The incidence of apparent endometrial fragments in the vessels in the tunica muscularis was 4.5% (13 of 290 patients). The incidence was higher in lymphatic vessels than in blood vessels. All of these intravascular fragments consisted of glandular and stromal cells in all patients and were found attached to the vascular wall in most of them (Fig. 4).

Lymphatic drainage in the muscle layer, the subserous layer, and out of the uterus

Lymphatic drainage in the endometrial and muscle layers. The ink flowed diffusely into the connective tissue spaces of the superficial endometrial layer and spread gradually into the vessels of the middle endometrial layer. In the muscle layer the staining became more intense, and clearly delineated capillary lymphatic vessels were observed (Fig. 5). Lymph flow through the muscle layer drains toward the subserous lymph vessels. The subserous lymph in front and back of the uterus flows to bilateral sites.

Subserous lymphatic channels and drainage. These channels showed an irregular, thick, netlike distribution. The valves of the lymphatic vessels were observed. Oak leaf–like endothelial cells became visible when ink mixed with silver nitrate solution was used (Fig. 6).

The most interesting finding related to lymphatic drainage was a direct stream from the uterus to the

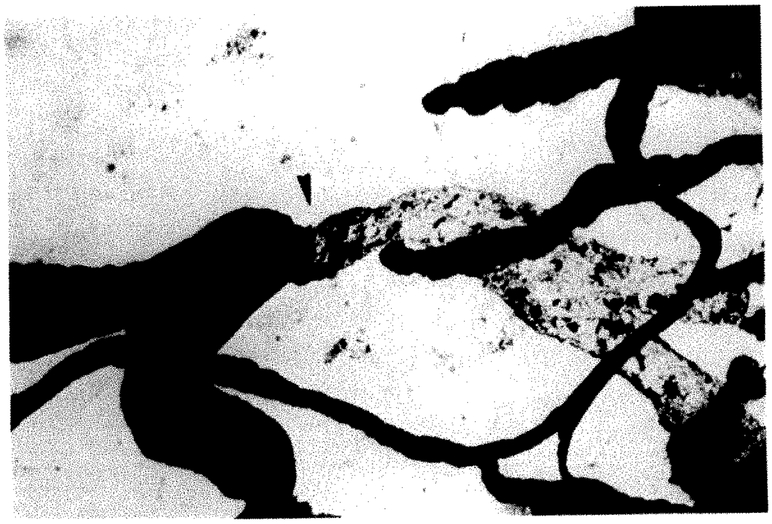

Fig. 6. Distribution of lymphatic channels and drainage in subserosa (India ink mixed with silver nitrate solution technique). *Arrow* shows site of valve.

ovaries. We confirmed the existence of this direct stream from certain areas in the uterine fundus to the ovaries by this ink method (Fig. 7).

Lymphatic drainage out of the uterus. Lymphatic drainage out of the uterus is shown in Fig. 8. The ink flowed from the uterus into the tubes, ovaries, and round ligaments. Flow from the fundus to the infundibulopelvic ligaments was identified, as well as flow from a wide area in the vicinity of the cervix reaching the cardinal ligaments. Part of the drainage to the cardinal ligaments appeared to flow back to the bladder. In the back of the uterus, lymph flowed out in the same way. At the boundary of the uterosacral ligaments, drainage appeared to be extremely complicated. On the basis of these findings and those obtained from evaluation of sites of endometrial cancer metastasis (unpublished data), routes through which lymph may flow out from the uterus were postulated (Fig. 9). The following five routes were postulated: (1) from the uterus into the internal and common iliac and aortic lymph nodes via the pelvic infundibular ligaments, (2) from the obturator, internal, and external iliac lymph nodes to the common iliac lymph nodes via the cardinal ligament, (3) to the inguinal lymph nodes via the round and umbilical ligaments, (4) directly into the ovaries, and (5) directly into the fallopian tubes.

Intrauterine lymphatics in the tunica muscularis uteri. Intrauterine lymphatics were distributed in the lamina propria mucosa, myometrium, and subserous layer and showed a variety of shapes and calibers. The lumina of lymphatic capillaries were filled with drak amorphous material in contrast to the lucent lumina of blood capillaries. Lymphatic endothelial cells were thin and flat on the luminal surface, but small projections extending into the surrounding tissue, in which hemidesmosome-like apparatuses were present, were found at various places. The basal lamina was discontinuous or absent. The most interesting finding was the presence of openings between adjacent endothelial cells in patients with

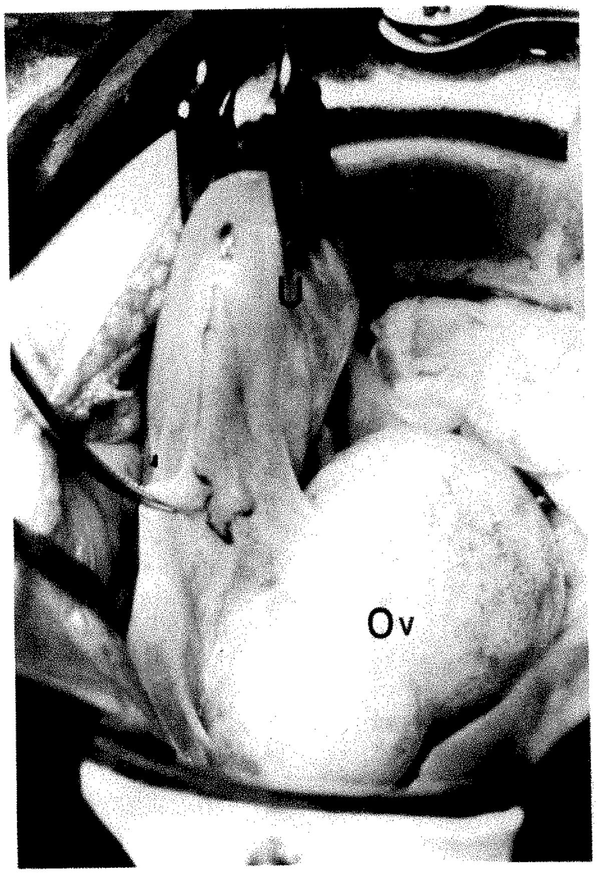

Fig. 7. Confirmation of direct lymphatic stream from uterus to ovary (India ink technique). *U*, Uterus; *Ov*, ovary.

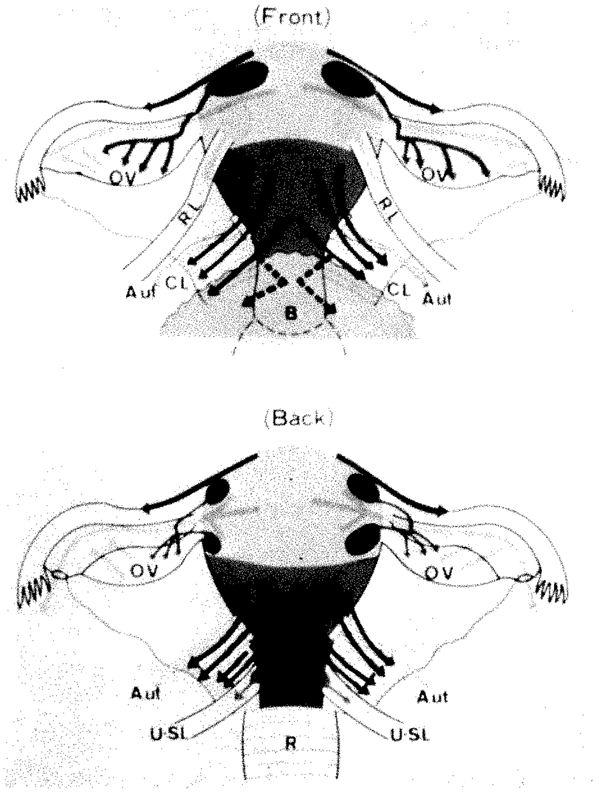

Fig. 8. Lymphatic drainage of human uterus.

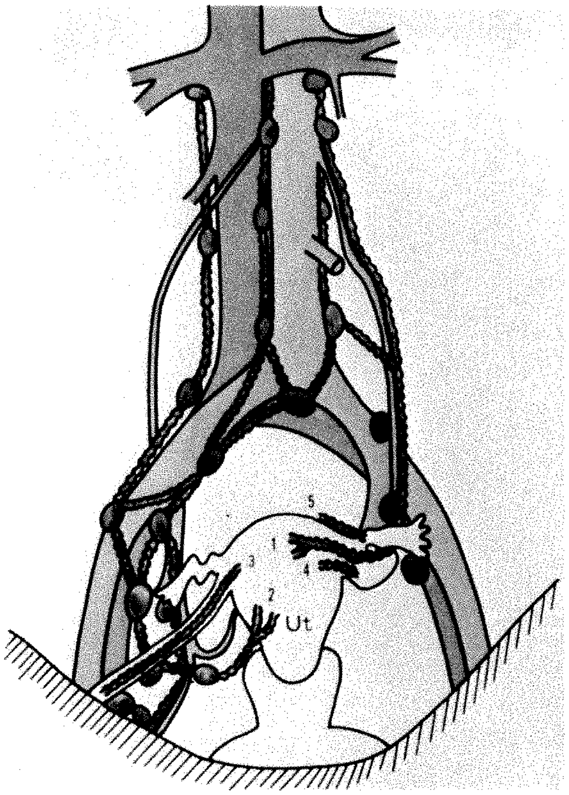

Fig. 9. Lymphatic drainage out of uterine body.

adenomyosis, inasmuch as these openings are not generally observed in lymphatic vessels under normal conditions (Fig. 10).

Lymphatic vessels in the muscle layer possessed valves in which the basal lamina was present beneath the endothelial cells (Fig. 11). The valves consisted of two endothelial cells. Lysosome-like bodies were occasionally present in the cytoplasm of these cells. The valves prevent reflux of the lymph and are involved in the transport of various substances.

Distribution of lymphatic vessels in the ovaries. India ink was found in the medullary lymphatic vessels, and ink flowed from the cortical lymphatic vessels on injection over the ovarian surface, resulting in profuse distribution. Lymphatics were abundant in the corpora lutea and were also observed in the theca folliculi externa of mature follicles and tunica albuginea.

The distribution of lymphatic vessels in the ovaries within the cortex was compared with that of the medulla; also lymphatic vessels in premenopausal and postmenopausal women were compared (Fig. 12). Lymphatic vessels in both the cortex and medulla were thick and abundant in reproductive-aged women but became narrow after menopause.

Comment

Cullen[1] reported that uterine adenomyosis occurs as a result of continuous infiltration of the endometrium into the muscle layer. Data obtained some years ago confirmed these findings.[13]

It has also been demonstrated that other factors related to the histogenesis of adenomyosis include dilatation of connective tissue spaces, increases in the number of reticular fibers, and increases in intrauterine pressure as a result of menstruation, pregnancy, delivery, abortion, and curettage.[13]

One of the most important points related to histogenesis of external endometriosis is that external lesions are morphologically and functionally very similar to the basal layer of the normal endometrium (Fig. 1). In fact, most external lesions appear to derive or metastasize from the endometrium.

The question is through which routes does metastasis occur. One route is that followed by serous endometriosis. In this instance, which is often observed in daily clinical practice, adenomyosis spreads over the muscle layer and reaches the serous membrane. This pattern, which is the result of continuous (nonvascular) invasion of endometrial fragments into the muscle layer spaces, was seen in 14.3% of 14 patients examined. Microscopically continuous (42.9%) or partially intermittent (14.3%) lesions were observed in muscle layer spaces between the adenomyosis lesions and the corresponding superficial lesions.

The incidence of serous endometriosis, which derives from adenomyosis continuously or semicontinuously,

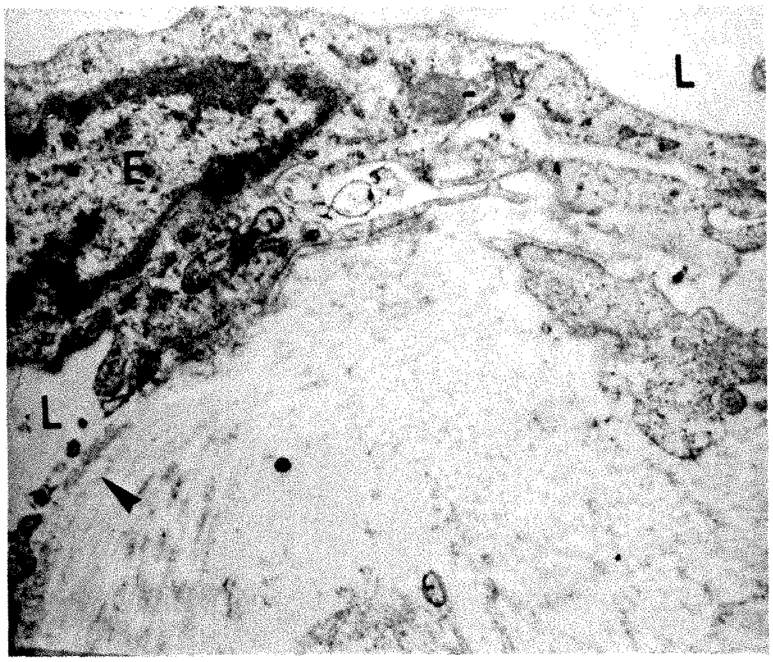

Fig. 10. Electron micrograph of lymphatic capillary. Part of lymphatic capillary shows opening *(arrow)* between adjacent endothelial cells. *L*, Lumen of lymphatic capillary; *E*, endothelial cell of lymphatic capillary.

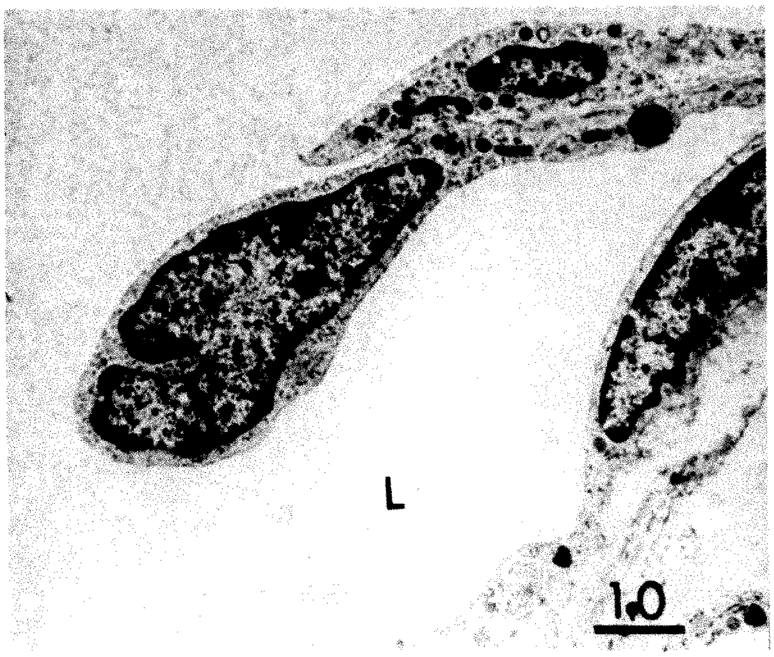

Fig. 11. Electron micrograph of lymphatic vessels showing part of valve of lymphatic vessel in muscle layer of uterus. Valve consists of two endothelial cells. *L*, Lumen of lymphatic vessel.

is thought to be quite high if that derived from the endometrium is also included.

The transvascular implantation theory advocated by Halban[10] is another example. In the present study endometrial fragments were detected in the lymphatic vessels of the muscle layer in 4.5% of patients. Endometrial fragments were found floating in the lymphatic vessels in only a few patients. In most instances part or approximately one half of the endometrial fragment surface was in contact with the wall of lymphatic vessels,

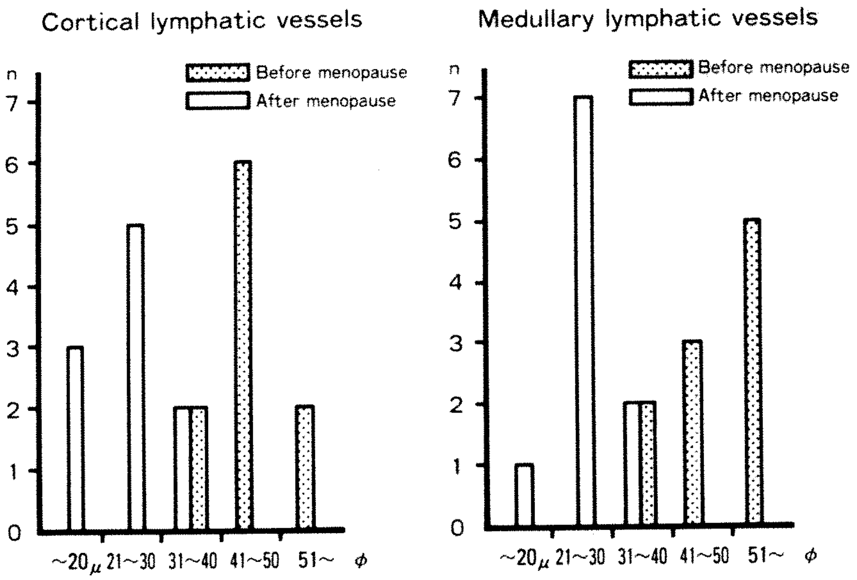

Fig. 12. Distribution of lymphatic vessels in ovaries.

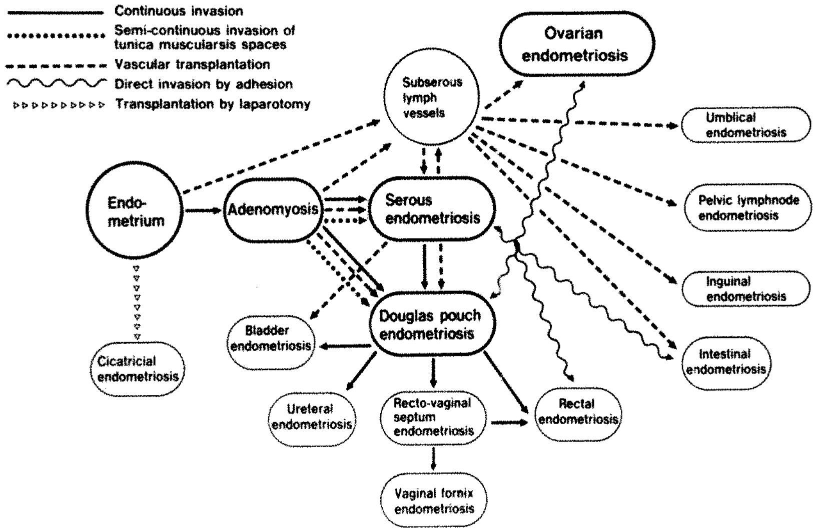

Fig. 13. Diagram showing histogenesis of endometriosis.

giving the impression that these fragments moved forward by changing their shape on the wall to which they closely adhered. This process needs to be studied further to obtain more insight into how an endometrial fragment or a cancer cell metastasis occurs.

As shown in Fig. 10, endothelial cells of capillary lymphatic vessels are opened by changes in tissue pressure. Endometrial fragments and cancer cells may enter the lymphatic vessels from these openings.

Only a few studies have been conducted on lymphatic vessels and capillary lymphatic drainage in the uterus and their microstructure.[14] This study on lymphatic drainage conducted with India ink showed that the ink was absorbed into the endometrium from the intrauterine cavity by entering vessels that seemed to be capillary lymphatic vessels from connective tissue spaces in the endometrial layer. The ink flow gradually became thicker and entered the lymphatic vessels in the muscle layer and then proceeded into the network of abundant large lymphatic vessels under the serous membrane. This flow seemed to have five routes: pelvic infundibular ligament, lymphatic vessels in the cardinal ligament, ovaries, fallopian tubes, and round ligament. The first two would be the main routes. It is interesting that these routes are consistent with the sites and incidence of metastasis of endometrial cancers.

With regard to the relationship between lymph flow into the ovaries and serous endometriosis, the lymph flow into the ovaries may occur readily through part of the uterine body. The influx may occur from vessels at the ovarian hilus and the adjacent cortical areas. Ovarian and serous types of endometriosis were very closely related not only to each other but also to adenomyosis; serous endometriosis and adenomyosis seem to be involved in the development of ovarian endometriotic lesions.[15] Transvascular implantation seems to be the most likely mechanism involved in the pathogenesis of ovarian lesions.

In conclusion, on the basis of the preceding findings endometriosis would seem to be mainly the result of continuous or semicontinuous invasion and vascular transplantation of the endometrium (Fig. 13). Concerning ovarian endometriosis, other mechanisms besides endometrial transplantation might be involved in its pathogenesis; however, they were not referred to in this article.

The following factors seem to be of critical importance in elucidating the histogenesis of endometriosis in the future: (1) to further clarify the histopathologic definition of endometriotic lesions; (2) to determine whether endometriotic lesions derive only from the endometrium or from other places as well; (3) to remember that endometrial tissues (fragments) are able to either continuously or noncontinuously infiltrate nearby tissues; (4) to take note that flow of lymph and other body fluids in and out of the uterus cannot be neglected as possible pathways; (5) to compare the mechanisms of invasion of adenomyosis and endometrial cancer; and (6) to review the incidence of conditions that match the definition of pulmonary, subcutaneous, pelvic lymph node, and ovarian endometriosis.

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Peritoneal endometriosis: Endometrial tissue implantation as its primary etiologic mechanism

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The mechanisms involved in primary implantation of pelvic endometriosis were investigated in women undergoing laparoscopy as part of an infertility evaluation. Among the women with a diagnosis of endometriosis, those with no observed lesions in the ovaries and no adhesions in the pelvic cavity were classified as being in stage I. These women were further subdivided into two groups: the stage Ia group in which implants were found in one area (25 women) and the stage Ib group in which implants were found in two or more areas (29 women). In both groups the most frequent site was the uterosacral ligaments followed by the posterior uterine serosa, the posterior cul-de-sac, and the posterior broad ligaments in that order. Regardless of the position of the uterus, endometriosis was found significantly more frequently in the posterior compartment. The finding that the most frequent implants on the uterosacral ligaments were near the tubal ostium and, in the stage Ia group, the absence of implants on the anterior uterine serosa but a 33% frequency on the posterior uterine serosa, which is more frequently exposed to menstrual blood, suggest that implantation of endometriosis is the result of retrograde menstruation. However, the possibility that metaplasia of the serosal epithelium is stimulated by menstrual blood cannot be ruled out, inasmuch as the process of implantation in the peritoneum has not yet been proved. (AM J OBSTET GYNECOL 1991;165:210-4.)

Key words: Etiology, endometriosis, laparoscopy, retrograde menstruation

The location of endometrial implants is considered important in the study of the mechanisms of implantation of peritoneal endometriosis in the pelvic cavity. This article examines the validity of Sampson's[1] regurgitation theory by studying the distribution of the disease in patients in the early stage of peritoneal endometriosis in the pelvic cavity.

Material and methods

Of the patients visiting our infertility clinic in whom endometriosis was diagnosed by laparoscopy, those with implants only on the serosa in the pelvic cavity and with no adhesions or ovarian endometriosis were defined as being in the early stage of endometriosis (stage I). These patients were divided into two groups: the stage Ia group in which lesions were found in only one area (25 patients), and the stage Ib group in which lesions were found in two or more areas (29 patients). Biopsy samples were obtained from patients with typical findings such as blueberry spots and blood blebs. Furthermore, yellow-brown peritoneal patches and red flame-like lesions similar to those described by Jansen and Russell[2] were also investigated histopathologically.

Table I. Location of endometriotic lesions

Location	*Ia (%)*	*Ib (%)*	*All of stage I (%)*
Round ligament	0/25 (0)	5/29 (17)	5/54 (9)
Anterior uterine serosa	0/25 (0)	3/29 (10)	3/54 (6)
Posterior uterine serosa	7/25 (28)	20/29 (69)	27/54 (50)
Tube	0/25 (0)	5/29 (17)	5/54 (9)
Posterior cul-de-sac	5/25 (20)	11/29 (38)	16/54 (30)
Posterior broad ligament	3/25 (12)	10/29 (34)	13/54 (24)
Anterior broad ligament	0/25 (0)	0/29 (0)	0/54 (0)
Uterosacral ligament	10/25 (40)	20/29 (69)	30/54 (56)

Experimental endometriosis was induced as follows: A portion of the uterine serosa of a 10-week-old Sprague-Dawley rat (240 to 260 gm), was cut open and several 5 × 5 × 1 mm pieces of tissue were obtained, which included endometrium. The pieces of tissue were sutured onto two sites—the mesenterium and the retinaculum uterus—with a 4.0 nylon thread with the endometrium side toward the abdominal cavity. Three weeks after transplantation, laparotomy was performed to examine the transplanted tissue macroscopically and histopathologically.

Results

As shown in Table I, in the stage Ia group the most frequent site of endometriosis was the uterosacral lig-

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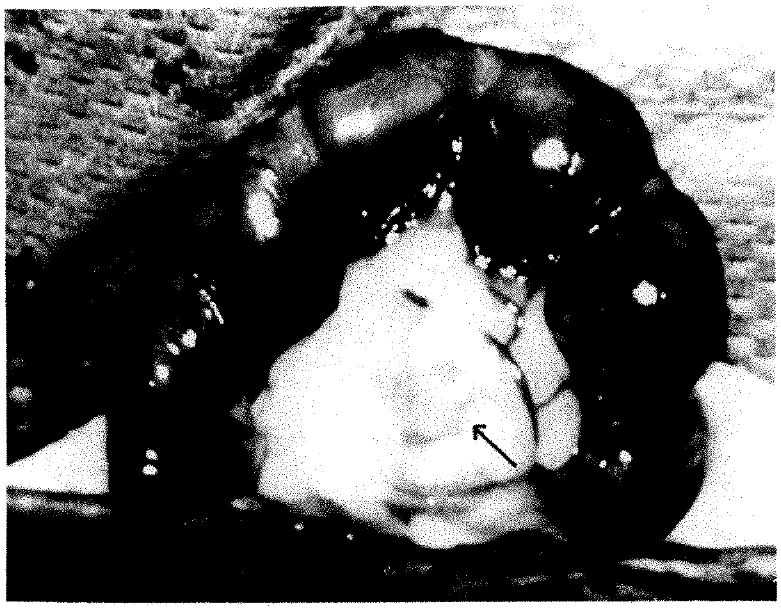

Fig. 1. Aspect of implants 3 weeks after surgery. *Arrow* indicates cystic change of implants.

Table II. Distribution of endometriotic lesions

Stage	*No. of patients*	*No. of patients with endometriotic lesions in anterior compartment*	*No. of patients with endometriotic lesions in posterior compartment*
Ia	25	0/25 (0%)	25/25 (100%)
Ib	29	9/38 (23.7%)	29/38 (76.3%)

Ia, Endometriotic lesions found in one area; *Ib,* endometriotic lesions found in two or more areas.

aments (49%), followed by the posterior uterine serosa (28%), the posterior cul-de-sac (20%), and the posterior broad ligaments (12%). In the stage Ib group both the uterosacral ligaments and the posterior uterine serosa were equally common sites (both 69%), and the posterior cul-de-sac was also a common site for endometriosis (38%).

When our study population was considered as a whole, the most common site of endometriotic lesions was the uterosacral ligaments (56%), followed by the posterior uterine serosa (50%), the posterior cul-de-sac (30%), and the posterior broad ligaments (24%). Implants were also found on the round ligaments (9%), the fallopian tubes (9%), and the anterior uterine serosa (6%), but they were not found on the anterior broad ligament. The preceding results were reexamined by dividing the pelvic cavity into two compartments. The anterior compartment consisted of the anterior cul-de-sac, the anterior broad ligaments, and the anterior uterine serosa. The posterior compartment included the posterior cul-de-sac, the posterior broad ligaments, the posterior uterine serosa, the uterosacral ligaments, and the sigmoid colon. In the stage Ia group implants were found only in the posterior compartment, whereas in the stage Ib group 76.3% of the implants were found in the posterior compartment and 23.7% in the anterior compartment (Table II). In the 38 (of 54) patients with primary infertility in both stage Ia and stage Ib groups, the most frequent site was the uterosacral ligament, followed by the posterior uterine serosa, and the posterior cul-de-sac.

With regard to the relationship between the position of the uterus and the location of the implants, 88.6% of patients with anterior uteri (38 patients) were found to have implants in the posterior compartment and 11.4% of them had implants in the anterior compartment. Among patients with posterior uteri (16 patients), 83.3% had implants in the posterior compartment and 16.7% had implants in the anterior compartment. In both positions implants were found much more frequently in the posterior compartment. There were no patients with implants in the anterior compartment only.

The results of the experiments in rats were as follows: On the sutured part, a unilocular cyst filled with transparent fluid was observed (Fig. 1). On examination of the tissue, a glandular structure was observed on the inner surface of the cyst, and the intraglandular space was filled with fluid and the presence of active glands noted. The glandular structure was covered with

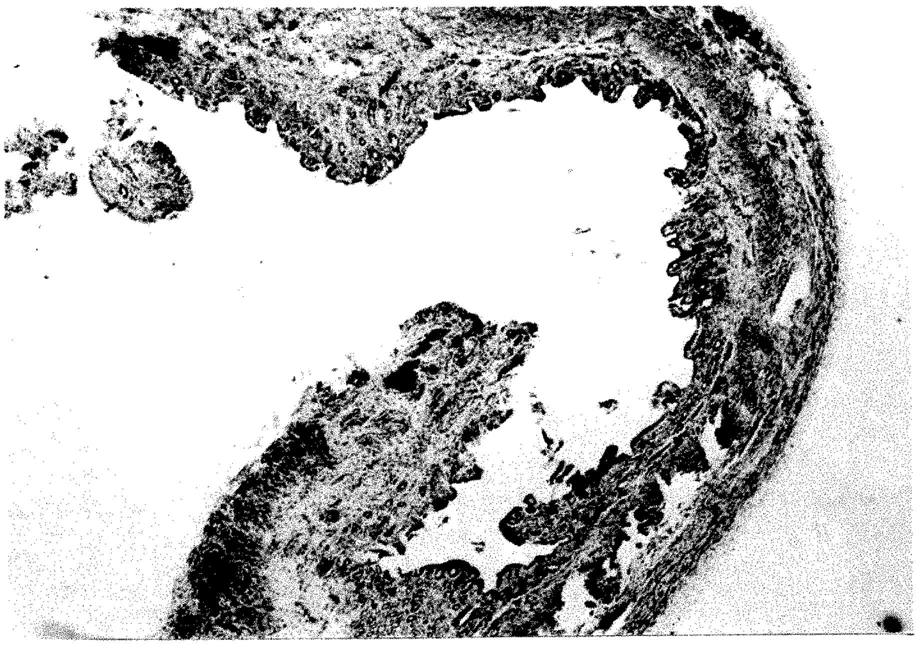

Fig. 2. Glandular structure covered by simple epithelium and connective tissue.

simple epithelium and connective tissue—the intact serosa (Fig. 2). Many blood vessels were observed around the glandular structure.

Comment

We found that in patients with pelvic endometriosis affecting the serosa only and with no adhesions, endometriotic implants were located much more frequently in the posterior compartment compared with the anterior compartment. Particularly in patients with lesions in only one area (stage Ia), implants were found in the posterior compartment only. Jenkins et al.,[3] who conducted similar investigations, reported that in those patients with a severely anteflexed uterus, implants were found in the anterior compartment only. In our previous study in which lateral-view hysterosalpingograms were used, we found that in patients with a retroflexed uterus, most of the contrast medium 24 hours after administration had accumulated in the posterior compartment (unpublished observations). In patients with an anteflexed uterus, the contrast medium diffused into both compartments, but the amount was larger in the posterior compartment. This indicates that regardless of the position of the uterus, except in patients with a severely anteflexed uterus, menstrual blood would accumulate in the posterior compartment centering around the posterior cul-de-sac. In other words, the finding that in the earliest stages of the disease endometriotic implants are seen more frequently in the posterior compartment means that implantation is likely to occur where menstrual blood has accumulated. Considering the location of the tubal ostium, which is situated close to the point of insertion of the uterosacral ligament, it is quite interesting to note that the most frequent site of implantation in incipient endometriosis is at the uterosacral ligaments. The finding that in the stage Ia group the implantation frequency in the anterior uterine serosa was 0% compared with 33% in the posterior uterine serosa, which is more frequently immersed in menstrual blood, seems to support Sampson's implantation theory. Theoretically, suitable sites for transplantation occurring as a result of retrograde menstruation are: (1) sites immersed in menstrual blood, (2) sites likely to be influenced by gravity, and (3) fixed organs. Actual implant locations as reported here corresponded to these possible sites.

However, several problems have been pointed out in relation to Sampson's theory. First, can endometrial particles go through the uterine tubes? Second, can endometrium that is cast off at the time of menstruation survive and have the ability to grow elsewhere within the pelvic cavity? Third, serosal lesions are located at a certain depth under the serosa and are always covered with healthy serosa. This suggests a transvascular migration mechanism, which supports the theory of lymphatic and vascular metastasis.

In response to the first question, the presence of endometrial fragments in the isthmic portion of the tubal lumen has already been demonstrated.[4] Endometrial cells and endometrial fragments are often found in hemorrhagic ascites collected during laparoscopy conducted at the time of the menstrual period

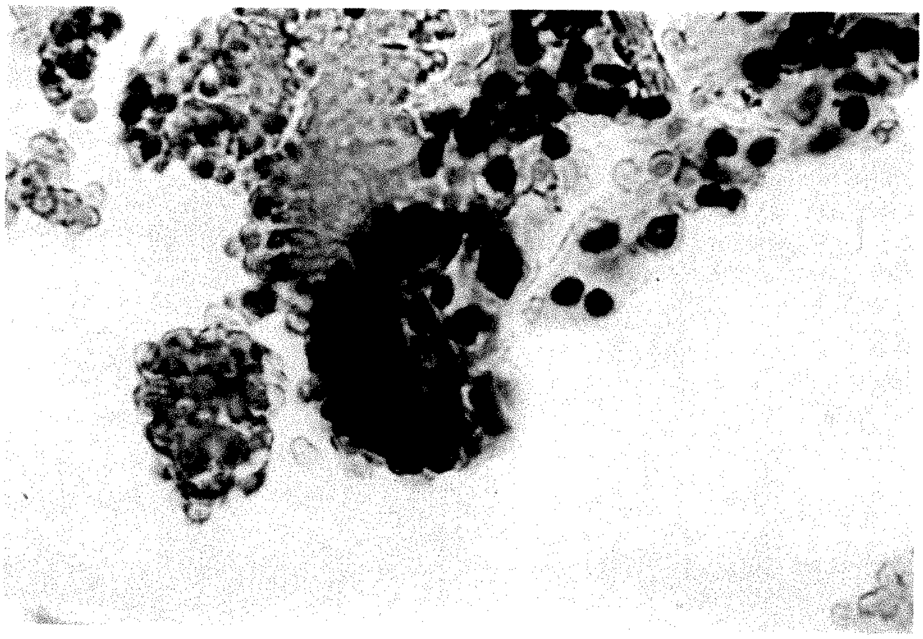

Fig. 3. Endometrial fragments in abdominal fluid obtained on fourth day of menstruation.

(Fig. 3). These facts prove that the endometrial fragments usually pass through the fallopian tubes and are discharged into the abdominal cavity.

In response to the second question, in vitro cultivation and transplantation experiments of endometrium at the time of menstruation have proved that cast-off endometrium has the ability to grow at ectopic locations.[5] In this regard results of the experiment by Te Linde and Scott[4] are very interesting. Te Linde and Scott[4] performed laparotomy on *Macacus rhesus* monkeys. They made a cut at the cervix, or in some instances at the vagina, then turned the uterus 180 degrees so that the opening of the cervix was toward the head, and then fixed it. This way the menstrual blood flowed around the sigmoid colon and into the abdominal cavity. After a certain period of time, they performed a second laparotomy and reported the presence of endometrial implants in the sigmoid colon and the cervix. On the other hand, Ridley and Edward[6] injected endometrial fragments found in human menstrual blood into the abdominal fascia above the symphysis pubis at the midline and found endometriosis at the injection site 175 days later.

Thus the viability and transplantability of endometrial fragments in the menstrual blood have been well documented.

In response to the third question, the discussion is based on the results of our experiments in rats. The endometrium transplanted on the mesenterium or the retinaculum uterus was covered by the intact serosa. The results of this experiment demonstrate that serosal lesions covered by healthy serosa do not necessarily arise from vascular metastasis, but they can also develop after implantation of endometrial fragments on the surface of the serosa. Rock et al.[7] conducted a histologic study with the use of 50 fallopian tubes cut off at the proximal segment from 25 patients who desired a reversal operation 1 to 4 years after tubal sterilization. They reported the presence of endometriosis at the tip of the proximal segment of the ligated tube. Some patients also had fistulas at the tip and had endometriotic implants at those fistulas. In these 25 patients no endometrial implants were observed on the serosa in the pelvic cavity or the ovaries. Two other facts support Sampson's theory. First, endometrial implants are frequently found at sites such as the fimbria, where menstrual blood regurgitates into the abdominal cavity. Here perifimbrial adhesions occur frequently, and their morphologic structure allows debris from menstrual blood to adhere easily. Second, in many patients with endometriosis tubal patency is preserved. However, we have neither found nor read any evidence proving endometrial transplantation to the serosal surface of the pelvic cavity. On the other hand, endometrial implants in the pelvic cavity of patients with primary amenorrhea or endometrial hypoplasia[8] have been reported, and some of these implants can only be explained by the coelomic metaplasia theory. Furthermore, there is a mesenchyme with multipotential properties just below the mesothelium that originates from the coelomic epithelium. The presence of this tissue satisfies the concept of the secondary müllerian system theory.[9] The peritoneum has the potentiality to be transformed into tissue that bears strong similarities to the müllerian duct, and it is possible that metaplasia at the serosal epithelium occurs when it is stimulated by menstrual blood and it develops further into endometriotic lesions. Notwithstanding these findings, which speak against Sampson's theory, the results presented here, as well as those of other reports in the literature, constitute strong evidence in favor of this theory. Implantation of endometrial tissue that reaches the pelvic cavity as a result of retrograde menstruation would be the primary etiologic mechanism of pelvic endometriosis.

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A reappraisal of the coelomic metaplasia theory by reviewing endometriosis occurring in unusual sites and instances

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Endometriosis occurring in unusual sites and instances was reviewed for the purpose of exploring the pathogenesis of the disease. Endometriosis is frequently observed in the pelves of menstruating women. It occurs in teenagers even before menarche, in women who have never menstruated, and in postmenopausal women. It affects not only the pelvic organs but also the right-sided thoracic organs and rarely the extremities. It occurs in men who are castrated and treated with estrogen. The affected sites in both common and uncommon endometriosis include cells arising from the coelomic membrane and sites exposed to metaplasia-inducing substances such as estrogen and unknown factors liberated from the degenerating endometrium. This appears to strengthen the coelomic metaplasia theory, although additional basic and experimental data must still be accumulated to confirm this theory. (AM J OBSTET GYNECOL 1991;165:214-8.)

Key words: Endometriosis, pathogenesis, coelomic metaplasia

Endometriosis is an enigmatic disease that affects women of reproductive age. It causes pelvic pain, dysmenorrhea, dyspareunia, and infertility. Its prevalence is reported to be 4.5% to 33.3% in women admitted for evaluation and treatment of infertility, 4.5% to 21.2% in those admitted for pelvic pain, and 0% to 7.1% in those admitted for pelvic masses.[1] Since it is not rare and it causes rather severe clinical manifestations, endometriosis has been studied extensively, and many theories have been proposed to explain the pathogenesis of endometriosis. Two of these theories are currently thought to be the most plausible: the implantation theory and the coelomic metaplasia theory.

The implantation theory was presented by Sampson.[2]

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This theory postulates that viable endometrial tissue is regurgitated at the time of menses via the oviducts and is implanted on the peritoneal surface and the pelvic organs.

The coelomic metaplasia theory was proposed and supported by Iwanoff[3] and Meyer.[4] The theory states that endometriosis develops from metaplasia of cells lining the pelvic peritoneum. The theory was strengthened by the results of Gruenwald's[5] embryologic studies.

Endometriosis mainly affects pelvic organs.[6] It is also known to occur in remote regions such as the lung, pleura, diaphragm, intestine, gallbladder, kidney, ureter, and umbilicus and in the extremities; it can even occur in men, although such endometriosis is rare. In the present article such unusual cases of endometriosis will be reviewed, and the plausibility of the two major theories will be discussed.

Emdometriosis occurring in adolescent girls

There are numerous case reports of endometriosis occurring in adolescent girls.[7-25] It may occur in girls as young as 10 and 12 years of age.[9, 11, 21] Endometriosis occurring shortly after menarche is found frequently but not exclusively in young girls with müllerian anomalies causing outflow tract obstruction. Schifrin et al.[15] reported 15 cases of adolescent endometriosis verified at operation, six of which had müllerian anomalies and nine of which did not. According to their data, time intervals between menarche and surgery in the two groups were calculated to be 3.0 ± 1.9 and 6.6 ± 1.8 years (mean ± standard deviation), respectively, the former being significantly shorter than the latter ($p < 0.05$). Endometriosis is observed not only in girls with müllerian anomalies but also in those with normal pelvic anatomy. Goldstein et al.[21] diagnosed endometriosis by laparoscopy in 74 (52.9%) of 140 adolescent girls with intolerable pelvic pain of unknown origin, 66 (89.2%) of whom had normal pelvic anatomy. Bullock et al.[16] diagnosed endometriosis in 9 (50%) of 18 adolescent girls with incapacitating dysmenorrhea, but pelvic anatomy was not described.

Outflow tract obstruction enhances transtubal regurgitation of menstrual flow, which contains endometrial debris and other known and unknown factors. The fact that endometriosis occurs rather frequently in girls with outflow tract obstruction and if it occurs it becomes clinically evident earlier in these girls than in those with normal pelvic anatomy may be important and suggestive of the pathogenesis of endometriosis. This is sometimes regarded as evidence that strengthens the implantation theory.

However, the implantation theory has been challenged. Clark[9] reported a 16-year-old girl who underwent laparotomy because of uncontrollable dysmenorrhea and in whom endometriosis was found to be developing in the left cornu of a bicornuate uterus. The girl had had pain in the lower quadrant of a monthly cyclic nature and of 3 to 4 days' duration since 8 months before menarche. Menarche at 12 years of age was accompanied by the same pain noted each of the previous months, but the pain was slightly more intense. Thereafter the pain became more severe with each menses and medical treatment was ineffective, which compelled her to undergo laparotomy. In this patient the periodic pain starting before menarche suggests that endometriosis had already developed before the endometrium started to respond to ovarian steroids. El-Mahgoub and Yaseen[26] reported endometriosis in a 26-year-old woman who had never menstruated even after estrogen-progestin supplementation. Whitehouse[27] and Seitz[28] described the occurrence of endometriosis in similar cases. None of these was associated with outflow tract obstruction. Development of endometriosis in these instances cannot be explained by the implantation theory.

Endometriosis occurring in the diaphragm, the pleura, and the lungs

Major symptoms of pleural/diaphragmatic endometriosis are catamenial pneumothorax, hemothorax, and chest pain. Slasky et al.[29] reviewed 63 cases of catamenial pneumothorax and noted the following constant clinical features: (1) Its temporal relation to menstruation. It occurs within 48 to 72 hours of the onset of menstrual flow and does not occur at other times in the menstrual cycle. (2) The right-sided predominance of the occurrence. The incidence of right-sided pneumothorax is approximately 90% in catamenial pneumothorax, whereas it is 57% in idiopathic spontaneous pneumothorax. (3) Its age distribution. It occurs in the third and fourth decades of life. Idiopathic spontaneous pneumothorax shows a peak incidence at a younger age. (4) The recurrent nature of the condition. Each episode of pneumothorax is associated with menses, although not all of menses are associated with pneumothorax. (5) Its relation to ovulation. Most reported instances were in parous women and none occurred during pregnancy. Suppression of ovulation may relieve the symptoms.

Twelve cases of histologically proved parenchymal pulmonary endometriosis have appeared in the literature, including one reported by us.[30-41] Clinical manifestations have included catamenial hemoptysis (6/12 cases), chest oppression (2/12), x-ray findings (3/12), and catamenial pneumothorax (1/12). It is noteworthy that 10 (83.3%) of the 12 patients had endometriotic foci in the right lung.

Hobbs and Bortnick[42] performed an experimental study to investigate the pathogenesis of pulmonary endometriosis. They excised endometrium from rabbits and injected the tissue into the ear vein of the same animals. Pulmonary endometriosis developed in 19 (79.1%) of the 24 rabbits treated. From this they proposed that hematogenous metastasis of the endometrium is the pathogenic mechanism of pulmonary endometriosis.

The right-sided predominance of both pleural/diaphragmatic and pulmonary endometriosis, however, could not be explained by the theory of hematogenous metastasis. Allen[43] stated that particles less than 22.5 μm enter into diaphragmatic lacunas in the peritoneal lining of the diaphragm. When this occurs regurgitated endometrial glands and stromal cells have a limited chance of passing through this physiologic sieve. Rosenshein et al.[44] documented that when ^{32}P is injected intraperitoneally, nearly all of the injected material passes through the right diaphragm or liver capsule. Merrill[45] demonstrated that degenerating but not intact endometrium liberates a metaplasia-inducing substance that passes through a Millipore filter. It is well known that peritoneal fluid circulates in a clockwise direction, running down the left peritoneal gutter, across the pelvic floor, up the right peritoneal gutter, and to the right diaphragm. This current transports small particles such as estrogen and a metaplasia-inducing substance to the right diaphragm and subsequently to the right pleural cavity. Since the pleura arises from the coelomic epithelium, the same as the peritoneum and the müllerian ducts, it might have the potential to develop into endometriosis under stimulation by metaplasia-inducing substances. In this way the occurrence of pleural/diaphragmatic and parenchymal pulmonary endometriosis and its right-sided predominance could be better explained by the coelomic metaplasia theory.

It is debatable, however, whether regurgitated endometrial glands and stromal cells pass through the diaphragm and are implanted on the pleura, since there may be cribriform fenestrations in the diaphragm that are more predominant in the right than in the left diaphragmatic leaf. Foster et al.[46] reviewed 41 cases of surgically treated catamenial pneumothorax. Pleural/diaphragmatic endometriosis was diagnosed in 32 cases, 27 (84.4%) of which were without diaphragmatic fenestrations and only five (15.6%) of which had fenestrations. Slasky et al.[29] reviewed 48 cases of catamenial pneumothorax in patients undergoing thoracotomy. They found diaphragmatic defects in 16 (33.3%), pleural/diaphragmatic endometriosis in 17 (35.4%), and both diaphragmatic defects and endometriosis in nine (18.87%). The implantation theory could be applied to explain the pathogenesis of thoracic endometriosis if diaphragmatic defects were found in the majority of cases. However, since this was not the case, it is difficult to accept the implantation theory as far as the pathogenesis of thoracic endometriosis is concerned.

Endometriosis occurring in the extremities

Although it is extremely rare, it is well known that endometriosis occurs in the extremities.[47-52] In the reported cases local pain occurred or became worse at menses. In all instances the tissues excised were histologically characteristic of endometriosis.

Gruenwald[5] introduced the coelomic metaplasia theory to explain the occurrence of endometriosis in the extremities. The mesenchymal limb buds develop adjacent to the coelomic epithelium during early embryonic development when the coelomic epithelium is still differentiating into various cell types. There is a good chance for the coelomic membrane–related cells to be misintegrated into the limb buds. The misintegrated coelomic cells possess the potential to develop into endometriosis under stimulation by metaplasia-induced substances such as ovarian steroids. There is no other reasonable and plausible explanation for the occurrence of this rare type of endometriosis.

Endometriosis occurring in men

Four cases of endometriosis occurring in men have been reported, three in the urinary bladder and one in the abdominal wall.[53-56] All were in older men with carcinoma of the prostate treated with local resection, orchiectomy, and long-term, high-dose estrogen therapy. Recurrent episodes of hematuria were noted in all three cases of endometriosis of the bladder, two of which showed polypoid lesions on cystoscopy and one with an ulceration. The histologic findings of the resected specimens were characteristic of endometriosis. Symptoms subsided only when estrogen therapy was discontinued. It is obvious that the endometriosis in these cases cannot be explained by the implantation theory. It is thought that these cases provide evidence supporting the coelomic metaplasia theory.

Conclusion

With regard to the occurrence of endometriosis in unusual sites and instances, the coelomic metaplasia theory provides the most reasonable explanation. The coelomic metaplasia theory could also explain the occurrence of endometriosis in the pelves of adult women. It is not justifiable to discriminate these two types of endometriosis in the discussion of its pathogenesis. Wherever it occurs the affected sites include cells arising from the coelomic membrane that are exposed to metaplasia-inducing substances such as estrogen and a factor liberated from degenerating endometrium (Merrill factor).[15] It is well recognized that the peritoneal fluid concentration of estradiol is as high as 300 to 1000 pg/ml during the late follicular and luteal phases of the ovulatory cycle. Transtubal regurgitation of menstrual blood is a common phenomenon. Menstrual blood is abundant in endometrial debris and therefore so is the Merrill factor. It is natural to assume that female pelvic organs are constantly exposed to such metaplasia-inducing substances, since human beings assume an upright position. This appears to be the reason why endometriosis mainly affects pelvic organs of women of reproductive age.

Because of a lack of supporting basic evidence, the coelomic metaplasia theory is sometimes criticized as follows: (1) Why does endometriosis occur exclusively in women? There are only four reported cases of endometriosis in men. Isn't male endometriosis attributable to estrogen stimulation of the prostatic utricle, a müllerian remnant in men? (2) Why does endometriosis mainly affect the pelvic organs? It should occur in any site where cells arising from the coelomic membrane are present. (3) Why does endometriosis occur almost exclusively in women of reproductive age? The frequency of epithelial metaplasia elsewhere increases with age. (4) Why does endometriosis almost inevitably occur in women with functioning endometrium?

However, these criticisms can be turned around: (1) Endometriosis does occur in men if their estrogen levels are overwhelmingly superior to negligible androgen levels.[53-56] (2) Endometriosis indeed occurs wherever coelomic cells are present. (3) Endometriosis occurs in prepubertal girls[9] and also in postmenopausal women.[57-61] Endometriosis occurs even in women who have never menstruated.[26-28]

Thus the coelomic metaplasia theory favorably explains the origin of endometriosis regardless of the sites, conditions, or instances of its occurrence. It is necessary, however, to accumulate more basic and experimental data to confirm the coelomic metaplasia theory.

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Secondary müllerian system and endometriosis

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The pathogenesis of endometriosis is controversial. The two principal histogenetic theories are (1) metastases of endometrial tissues to an ectopic location (metastatic theory) and (2) metaplastic development of endometrial tissue at the ectopic site (metaplastic theory). Studies on the development of the coelomic cavity and the müllerian duct, as well as expression of CA 125 during the fetal period and in the adult, strongly suggest that coelomic epithelium–related tissues and müllerian-derived epithelia of the adult have a shared embryologic ancestry. Many manifestations of müllerian-directed metaplasia suggest that tissues derived from the coelomic epithelium and its accomanying mesenchymal cells, referred to as the "secondary müllerian system," have the potentiality to differentiate into müllerian-directed epithelium and stroma. This metaplastic potentiality of the secondary müllerian system constitutes a basic concept in the pathogenesis of endometriosis. (AM J OBSTET GYNECOL1991;165:219-25.)

Key words: Secondary müllerian system, müllerian-directed metaplasia, CA 125, endometriosis

Endometriosis is one of the most commonly encountered diseases in gynecology and infertility clinics. However, this disease is an enigma because both its cause and pathophysiology remain poorly understood. It is known that ectopic endometrial tissue is sensitive to the estrogen produced during the menstrual cycle and that the disease regresses after menopause.

Endometriosis is defined as the presence of endometrioid epithelium and stroma in an ectopic site excluding the myometrium; ectopic endometrium within the myometrium is designated as adenomyosis.[1] Although adenomyosis has been referred to as endometriosis interna, this term is no longer used because endometriosis and adenomyosis are unrelated disorders.[1]

The pathogenesis of endometriosis is controversial. The two principal histogenetic theories are (1) metastases of endometrial tissues to an ectopic location (metastatic theory) and (2) metaplastic development of endometrial tissue at the ectopic site (metaplastic theory).[1] Some clinical investigators favor the metastatic theory,[2-4] but most pathologists prefer the metaplasic theory.[5-8] In addition, other investigators favor a combination of the metastatic and metaplastic theories, postulating that endometriosis represents a metaplastic response to the irritative effects of endometrial tissue that has metastasized to ectopic sites.[9]

Since Lauchlan[10] first used the term "secondary müllerian system" to refer to all müllerian-type epithelium (including endometriotic lesions) located outside the cavities of the original müllerian ducts, many manifestations of müllerian-directed metaplasia and nonneoplastic proliferations (both epithelial and mesenchymal), which can be identified on or immediately beneath the ovarian surface, the pelvic peritoneum, and the omentum and in retroperitoneal lymph nodes, have been reported.[1,8] This evidence has demonstrated the müllerian-directed metaplastic potential of these tissues and supports the metaplastic theory of endometriosis. In addition, common epithelial tumors of the ovary are considered to be derived from the surface epithelium covering the ovary and from the underlying stroma.[11] Although the ovary is not of müllerian origin, the source of these neoplasms, namely, the surface epithelium, is derived from the coelomic epithelium, which in the embryo gives rise to the müllerian ducts. The latter form the fallopian tubes, uterine body, cervix, and possibly the upper part of the vagina and their large variety of epithelia. Therefore the histologic heterogeneity of the müllerian-type epithelium encountered in this group of tumors does not contradict their common origin. The common histogenesis of epithelial tumors of the ovary has been accepted by most authorities[11]; however, the metaplastic theory does not seem to be widely accepted as the basic concept in the pathogenesis of endometriosis. Therefore, from an embryologic standpoint, the importance of the secondary müllerian system in the pathogenesis of endometriosis will be discussed here.

Embryology of the female genital tract

At the end of the second week of embryonic life, the human embryo is a bilaminar oval disk, approximately 0.2 mm in diameter. The two lamina are the first two

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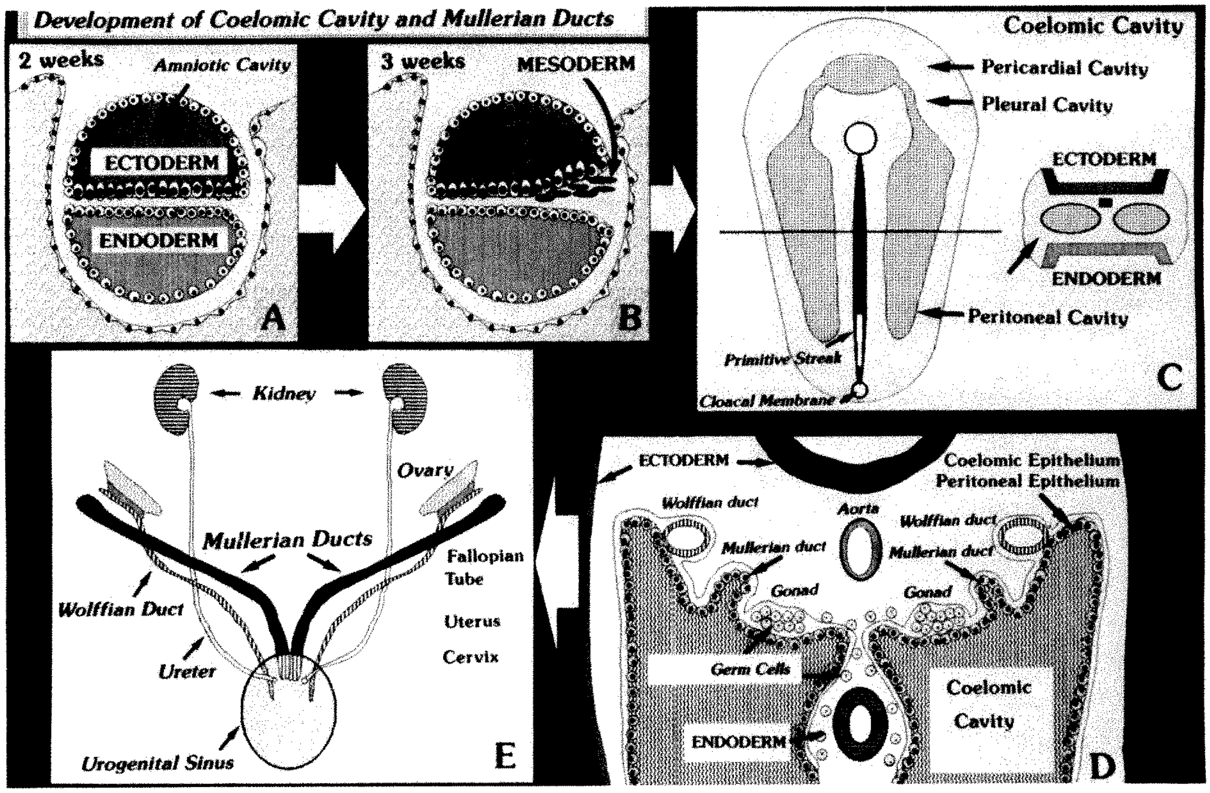

Fig. 1. Development of coelomic cavity and müllerian ducts during fetal period. **A,** Embryonic disc at second week of embryonic life. **B,** Embryonic disc at third week of embryonic life. **C,** Development of coelomic cavity. **D** and **E,** Development of müllerian ducts.

germ layers. The dorsal layer is the ectoderm and the ventral layer, the first to differentiate, is the endoderm (Fig. 1, *A*). The third embryonic layer, the mesoderm, is induced by the endoderm from the ectoderm during the third week of embryonic life (Fig. 1, *B*). The mesoderm continues to proliferate, separating the ectoderm and the endoderm. By the end of the third week of embryonic life, an intramesodermal cavity develops in the lateral portion of the mesodermal layer on both sides of the disk. These bilateral intramesodermal cavities extend cephalad producing a continuous intramesodermal canal that extends along the anterior portion of the embryo. The mesodermal canal forms a cavity termed the coelomic cavity, which is destined to become organized into the pericardial, pleural, and peritoneal cavities (Fig. 1, *C*).[12,13] The coelomic cavity is lined by flat mesothelial cells that have epithelial-like features but have differentiated from the underlying stellate stromal cells.[12] The coelomic epithelium, that is, the peritoneum, is therefore a mesothelial epithelium that is derived from the mesenchyme immediately beneath it.[12] This raises the possibility that there is more intimate biological relationship between the epithelium and the stroma in the mesodermal system than is the case where ectodermal or endodermal epithelia are supported by mesodermal stroma.[12]

By the end of the fourth week, on each side of the posterior wall of the coelomic cavity, a vertical ridge (urogenital ridge) develops. Late in the fifth to sixth week of embryonic life, on the lateral surface of the urogenital ridge, the coelomic epithelium invaginates at several points and coalesces to form a tube termed the müllerian or paramesonephric duct (Fig. 1, *D*).[12,13] The duct extends caudally in the urogenital ridge immediately lateral to the wolffian duct. The paired müllerian ducts give rise to the fallopian tubes, the uterus, the cervix, and the upper vagina (Fig. 1, *E*).[12,13]

The müllerian duct, which is composed of a single layer of undifferentiated columnar epithelium supported by fibroblastic mesenchymal cells, gradually differentiates into ciliated columnar epithelium in the fallopian tube, endometrial glandular epithelium and stromal cells in the endometrium, and mucin-producing cells in the cervix. Ultrastructurally the undifferentiated columnar epithelium gradually accumulates a large amount of glycogen particles until 18 weeks' gestation. These glycogen particles disappear at 20 weeks; then the ciliated cells in the fetal fallopian tube and the secretory cells in the endometrium and cervix are identified.[14] Thereafter adult histology of tubal, endometrial, and endocervical epithelium[15] is recognized in the respective parts of the female genital tract. Therefore, as a result of local differentiation, the

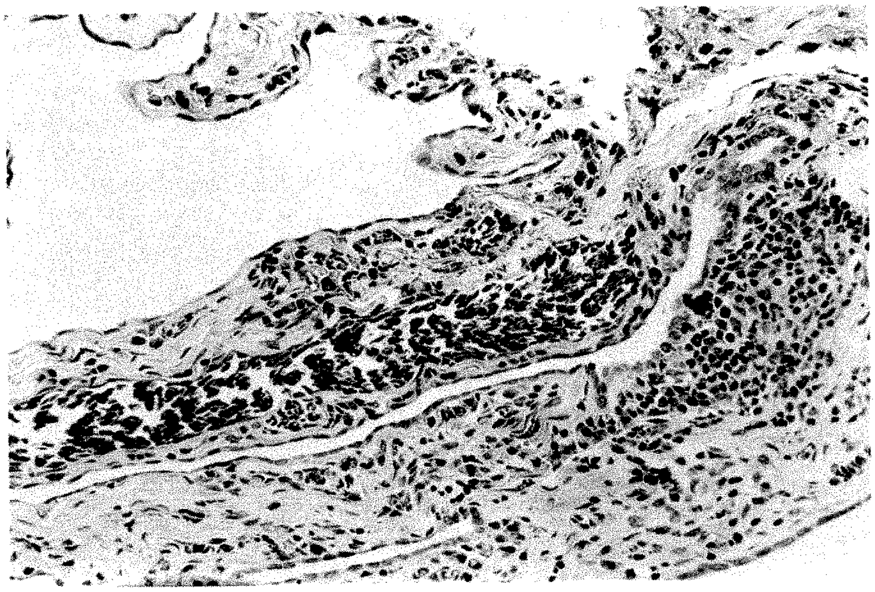

Fig. 2. Photomicrograph of omentum demonstrating increased cellular height of mesothelium. (Hematoxylin and eosin; original magnification ×200.)

differing adult histology of the tube, endometrium, and endocervix occurs.[14, 15] The mesenchymal cells and the müllerian epithelium also gradually differentiate into smooth muscle cells and endometrial stromal cells, resulting in the formation of the tubular muscular structure of the uterus and fallopian tubes.[16-18]

Localization of CA 125 in embryonic and adult tissues

CA 125 is an antigenic determinant recognized by the monoclonal antibody OC 125 against an ovarian cancer cell line (OVCA 433).[19] An assay of serum CA 125 has proved useful in the diagnosis and monitoring of patients with ovarian cancer.[20] However, serum CA 125 levels increase not only in women with other gynecologic malignancies[21] but also in women with benign conditions such as endometriosis[22] and pelvic inflammatory disease and during pregnancy.[23, 24] Immunohistochemically the CA 125 antigen is detected in coelomic epithelium and its derivatives such as the müllerian ducts of the fetus[25, 26] and müllerian-derived epithelia of the adult.[27, 28] More precisely CA 125 is present in fetal coelomic epithelium and its derivatives such as pleura, pericardium, peritoneum, ovarian surface epithelium, and müllerian ducts after 15 weeks' gestation. However, it is usually weakly positive for anti-CA 125 until 23 weeks' gestation.[25, 26] Thereafter CA 125 becomes strongly positive in müllerian-derived epithelia of the adult such as tubal, endometrial, and endocervical epithelium.[27, 28] Therefore CA 125 is considered a differentiation antigen associated with fetal coelomic epithelium and its derivatives, which are believed to be derived from the mesoderm of germ cells.[25, 26]

The expression of CA 125 during the fetal period and in the adult strongly suggests that the coelomic epithelium–related tissues (pleura, pericardium, peritoneum, and ovarian surface epithelium) and the müllerian-derived epithelia of the adult (tubal, endometrial, and endocervical epithelium) have a shared embryologic ancestry.

Secondary müllerian system

Awareness of the embryology of the peritoneal and müllerian duct epithelium led Lauchlan[10] to postulate that tissues derived from coelomic epithelium and its accompanying mesenchymal cells, referred to as the "secondary müllerian system," have the potentiality to differentiate into müllerian-directed epithelium and stroma.

Both on and immediately beneath the ovarian surface, the pelvic peritoneum, and the omentum, and in retroperitoneal lymph nodes, müllerian-directed metaplasia (both epithelial and mesenchymal) can be identified. The most common type of epithelial differentiation is tubal-type epithelium composed of ciliated cells without endometrial stromal cells, and this type is usually called endosalpingiosis.[29-32] In the omentum, when the mesothelium begins to appose, the cellular height of the mesothelium increases and shows a focal epithelioid architecture (Fig. 2). Thereafter the epithelium may invaginate and remain as an inclusion, and focal differentiation of the ciliated cells can be observed among the epithelioid cells (Fig. 3). If an inclusion of the ovarian surface epithelium is formed, the mesothelium-like ovarian surface epithelium gradually increases in cellular height and shows ciliated differentiation in the inclusion (Fig. 4). Endometrial-type epithelium with endometrial stroma is often observed

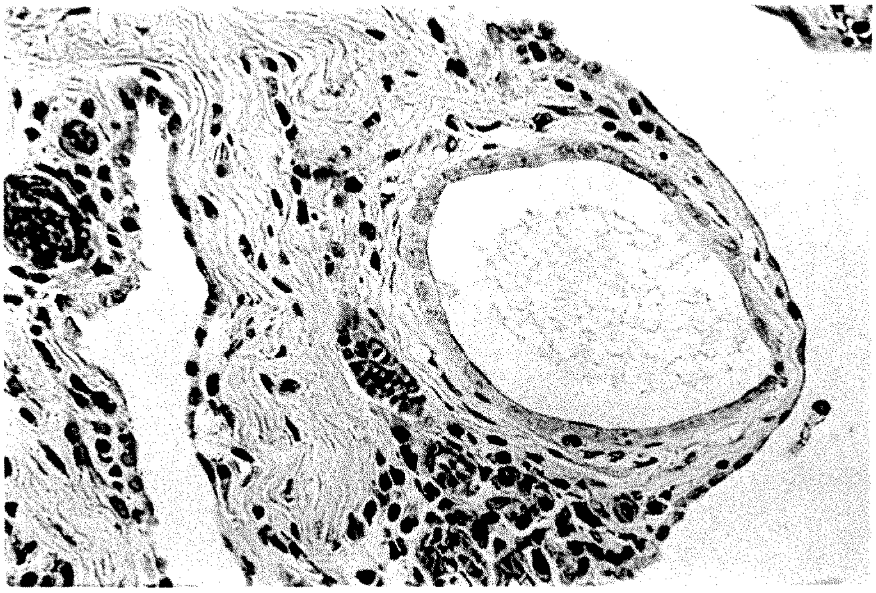

Fig. 3. Photomicrograph of omentum demonstrating glandular structure with focal ciliated differentiation. (Hematoxylin and eosin; original magnification ×400.)

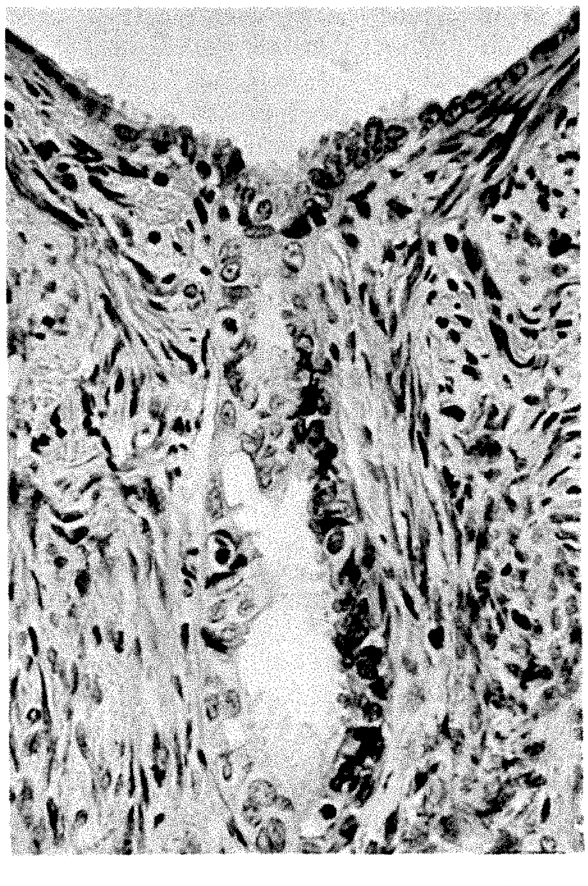

Fig. 4. Ovarian inclusion demonstrating gradual differentiation of surface epithelium into ciliated cells. (Hematoxylin and eosin; original magnification ×400.)

close to or intermingled with tubal-type inclusions. Endometrial-type epithelium without the appearance of endometrial stroma is also seen frequently. However, transformation of ovarian surface epithelium into endometrial-type epithelium with endometrial stroma and hemosiderin-laden macrophages can also occur (Fig. 5). Transformation of mucinous-type epithelium, although considerably less common,[1] occurs in the ovaries and in the retroperitoneal space.[33] In addition, adenomatoid tumor–like structures with ciliated or mucinous cells have also been observed in subperitoneal nodules experimentally induced by administration of sex steroids.[34]

The superficial stroma of the ovary is composed of the mesenchyme and the coelomic epithelium. This embryonic kinship allows the peculiar ability of the superficial ovarian cortex,[35, 36] the peritoneum,[37] and the uterine subserosa to undergo a decidual reaction during pregnancy in response to the prevailing hormonal milieu. Moreover, both the fallopian tube and the cervical stroma exhibit a focal decidual reaction during pregnancy, which is morphologically identical to that occurring in the endometrium. Furthermore, smooth muscle differentiation occurs in the peritoneum[38] and the superficial stroma of the ovary.[39] Smooth muscle cell differentiation is involved in leiomyomatosis peritonealis disseminata.[37, 38, 40, 41] Differentiation of the subcoelomic mesenchyme in the direction of smooth muscle, with the formation of multiple tumor-like nodules in the peritoneum including the ovarian surface, is the

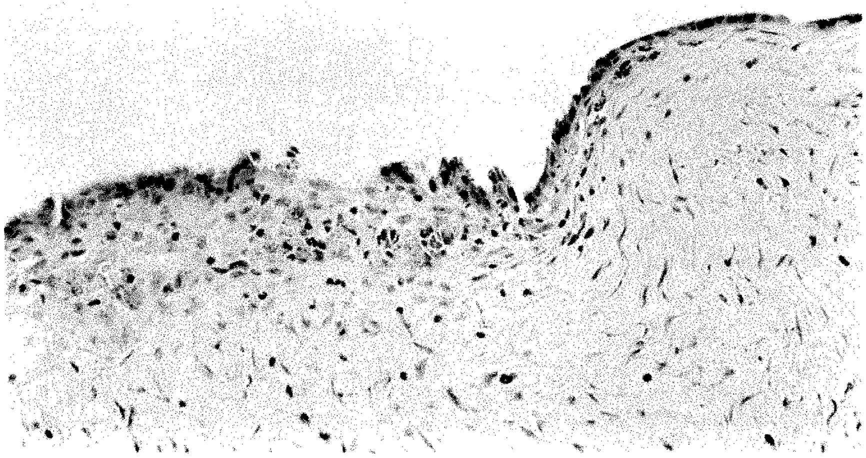

Fig. 5. Row of ovarian surface epithelium and endometrioid epithelium with endometrial stroma and hemosiderin-laden macrophages. (Hematoxylin and eosin; original magnification ×200.)

purported pathogenesis of leiomyomatosis peritonealis disseminata.[10, 11] Some degree of smooth muscle differentiation can sometimes be seen around inclusion cysts and endometriotic lesions.[39] The components of the latter are endometrial-type epithelium and stroma surrounded by smooth muscle resembling the architecture of the uterus.[39]

The incidence of tubal-type epithelium was assessed by Zinsser and Wheeler[30] who prospectively studied the omentum from surgical and autopsy material in both women and men; lesions were found only in surgically removed omenta from women (in 25.7% of cases) and were never identified in men. Müllerian-directed metaplasia is generally considered an acquired property; the lesions have not been described in premenarcheal girls.[42] It is likely that the lesions are induced by changes in the hormonal milieu that follow puberty. In addition to being hormonally induced, the lesions show variable hormone dependency and sensitivity. Müllerian-directed metaplasic lesions atrophy after menopause and may eventually degenerate and be resorbed.[30, 31]

Comment

Several reports in the literature support the concept of a metastatic theory of endometriosis.[1-4] Sampson[13] proposed that endometriosis is a consequence of reflux of endometrial tissue through the fallopian tubes by a process of retrograde menstruation, with subsequent implantation and growth on peritoneal surfaces.[13] Endometriosis within scars after uterine operations may be the result of iatrogenic implantation of endometrial tissue during surgery.[9] In addition, the presence of endometriosis in certain distant sites (e.g., lungs, skeletal muscle, subarachnoid space, and kidneys) is most easily explained by the theory of lymphatic and vascular metastasis.[14] Endometriosis within lymph nodes has also been explained as the result of lymphatic spread.

However, normal endometrium that can metastasize is essential for the metastatic theory to be valid. The metastatic theory cannot explain the finding of endometriosis in subjects in whom metastasis of normal endometrium could not occur or is highly unlikely, such as patients with Rokitansky-Kuster-Hauser syndrome, Turner syndrome, and pure gonadal dysgenesis who are amenorrheic and have hypoplastic uteri[15-17] and men with endometriosis of the bladder, prostate, scrotum, and abdominal wall.[18-51]

In most cases a process of retrograde menstruation may have an important role in the establishment of endometriosis. However, we cannot forget cases of endometriosis in which retrograde menstruation does not occur. Consequently the concept that coelomic epithelium–derived tissues have the potentiality to differentiate into müllerian-directed epithelium always lies a priori in the pathogenesis of endometriosis. On the basis of this concept, the influence of unknown factors that may be carried by a process of retrograde menstruation should be taken into account in the pathogenesis of endometriosis. The cases of leiomyomatosis peritonealis disseminata indicated that there is an individual sensitivity of subperitoneal mesenchymal cells to reproductive steroids.[41] Some cases of leiomyomatosis peritonealis disseminata occurred during pregnancy or use of oral contraceptives and regressed soon after delivery or discontinuation of drugs; but other cases, discovered in persons with normal ovarian cycles, regressed with castration.[41] Therefore individual sensitivity of coelomic epithelium–derived tissues to reproductive steroids should also be considered when trying to explain the pathogenesis of endometriosis. Indeed the metastatic theory is a fascinating one, but further research based on the metaplastic potentiality of the secondary müllerian system into müllerian-directed structures is necessary to elucidate the pathogensis of this enigmatic disease.

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Ultrastructural study of glandular epithelium in adenomyosis in comparison with those of proliferative endometrium and well-differentiated endometrial cancer

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Adenomyotic glandular tissue from five patients underwent electron microscopic investigation to observe its ultrastructural characteristics. The adenomyotic epithelium was compared with that of proliferative normal epithelium (two patients) and well-differentiated endometrial adenocarcinoma (two patients). The results revealed that morphologically the adenomyotic glandular epithelium is somewhat less differentiated than proliferative endometrium and that its cytoplasmic organelles have some similarities with those of endometrial cancer. Whether these similarities predispose the adenomyotic glandular tissue to malignant degeneration remains to be elucidated. (AM J OBSTET GYNECOL 1991;165:225-8.)

Key words: Adenomyosis, endometriosis, electron microscopy

The histogenesis of adenomyosis is still unknown at present. Ultrastructural observations of adenomyotic glandular epithelium would probably give some insight into its neoplastic characteristics[1]; consequently, I carried out electron microscopic studies to compare adenomyotic glandular epithelium with proliferative phase endometrium and well-differentiated endometrial adenocarcinoma.

Material and methods

Five patients with adenomyosis in the proliferative phase whose ages ranged between 30 and 52 years were selected for this study (Table I). Patients (cases 1 and 2) had surgery on the eighteenth and sixteenth days of the menstrual cycle, respectively. Both the orthotic and heterotopic glandular tissue were in the proliferative phase. Patients (cases 3, 4, and 5) had surgery during the secretory phase of the menstrual cycle. In these patients the endometrium was in the late secretory phase; however, the adenomyotic glandular tissue showed proliferative phase characteristics. Dating of tissue was carried out according to Noyes et al.[2] Well-differentiated endometrial adenocarcinoma tissue obtained from two other patients was also examined.

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Table I. Cases of adenomyosis studied

Case No.	*Age*	*Gravida/para*	*Chief complaint*	*Since LMP (days)*	*EM*	*Adenomyotic gland*
1	51	2/1	Irregular menses	18	Proliferative	Proliferative endometrium like
2	44	3/3	Hypermenorrhea	16	Proliferative	Proliferative endometrium like
3	52	4/2	Hypermenorrhea	30	25th day	Proliferative endometrium like
4	30	0	Hope for child	21	27th day	Proliferative endometrium like
5	39	4/3	Lower abdominal pain	52	27th day	Proliferative endometrium like; partly secretory endometrium like

LMP, Last menstrual period; *EM,* endometrium.

Electron microscopic findings in adenomyosis

Case 1. Nuclei were round with a smooth nuclear membrane and finely distributed chromatin. Nucleoli or chromatin clumps were not observed. The cytoplasm was either clear or dark. Mitochondria were round with some irregularities. Free ribosomes and the microfibrillar structure were moderately developed. The rough endoplasmic reticulum and glycogen granules were scarce. Extracellular structures, such as desmosomes, interdigitations, and microvilli, were relatively well developed.

Case 2. Nuclei were basically oval with a smooth nuclear membrane. Nucleoli, if present, had an irregular shape, and there was only one per cell. The chromatin was distributed diffusely. Free ribosomes were abundant, and the rough endoplasmic reticulum was moderately developed. Mitochondria were round with some irregularities. Glycogen granules were not found, and the extracellular structures were almost the same as in case 1.

Case 3. Nuclei were oval with a smooth nuclear membrane and fine chromatin. Nucleoli, if present, were smooth, and there was only one per cell. Mitochondria were oval with some irregularities. The rough endoplasmic reticulum, ribosomes, and the microfibrillar structure were moderately developed. Glycogen granules were scarce. Desmosomes were abundant, whereas interdigitations were poorly developed.

Case 4. Nuclei were oval with an extremely smooth nuclear membrane. If nucleoli were present, there was only one per cell, and they had a somewhat irregular morphologic appearance. Nuclear chromatin was finely distributed. The rough endoplasmic reticulum and free ribosomes were well developed. Mitochondria were oval with moderate irregularities. Lysosomes were not found and glycogen granules were scarce. The extracellular structures, such as microvilli, were relatively well developed.

Case 5. Nuclei were round with none or one nucleolus and a smooth nuclear membrane. Notably, the chromatin was attached to the nuclear membrane. The rough endoplasmic reticulum was scarce. Free ribosomes were moderately developed. Mitochondria were round with moderate irregularities. Lysosomes were abundant, but glycogen granules were not found. Interdigitations were strickingly abundant. The Golgi apparatus but not myelin body was found in all cells examined in the five cases studies.

Electron microscopic findings in proliferative phase endometrium

The proliferative phase endometrium of cases 1 and 2 was examined. The most common findings were as follows.

The cylindric epithelium was arranged tightly and piled up in some parts. The nucleoli were situated near the basement membrane and were clearly observed. Nucleoli were either rodlike or oval, with the chromatin scattered and attached to the nuclear membrane. Chromatin was present in moderate amounts. There was one smooth nucleolus and sometimes two. Mitochondria were rodlike or oval; the rough endoplasmic reticulum was elongated and moderately developed. Free ribosomes and lysosomes were also moderately developed. Glycogen granules were present in small amounts, and the Golgi apparatus was moderately developed. The microfibrillar structure was scarce, and the microvilli were developed but with slight length irregularities. Myelin bodies were not observed.

Electron microscopic findings in well-differentiated adenocarcinoma of the endometrium

Well-differentiated endometrial adenocarcinoma tissue from two other patients was also examined. In these tissues nuclei of an extremely irregular shape and with coarse chromatin were found. The rough endoplasmic reticulum was more abundant than proliferative endometrium or adenomyotic glandular epithelium. Free ribosomes were also abundant. Mitochondria were very irregular, especially the mitochondrial crista. Lysosomes were numerous and of different sizes. The Golgi apparatus was poorly developed. Microfibrillar structures were abundant, and myelin body was observed in some cells. Desmosomes and interdigitations were present, but they were not always observed. Microvilli were quite numerous and of various sizes.

Table II. Electron microscopic appearance of the nucleus in the three tissue categories studied

Nuclear	*Proliferative endometrium*	*Adenomyotic gland*	*Endometrial adenocarcinoma*
Shape	Rod to oval	Round to oval	Elongated
Chromatin	Scattered, adherent to nuclear membrane	Scattered, fine	Coarse
Nucleoli	1-2, regular	0-1, irregular	Increased, bizarre
Membrane	Slightly irregular	Extremely smooth	Very irregular

Table III. Electron microscopic appearance of the cytoplasm in the three tissue categories studied

Cytoplasmic	*Proliferative EM*	*Adenomyotic gland*	*Endometrial adenocarcinoma*
Rough ER	+ +	+ ~ + + +	+ + +
Free ribosomes	+ +	+ + ~ + + +	+ + +
Mitochondria			
Shape	Rod ~ oval	Round ~ oval	Irregular
Irregularities	+	+ ~ + +	+ + +
Lysosomes	+ +	− ~ + +	+ + +
Glycogen	+	− ~ +	+
Golgi apparatus	+	+	?
Microfibrillar structure	+	+ ~ + +	+ + +
Myelin body	−	−	+
Desmosomes	+ +	+ + ~ + + +	+
Interdigitations	+	+ ~ + + +	+ +
Microvilli	+ +	+ +	+ + +

ER, Endoplasmic reticulum; *EM*, endometrium; + + +, big amount of; + +, moderate amount of; +, small amount of; ?, not visualized.

Comparison of the adenomyotic glandular epithelium ultrastructure with that of the proliferative endometrium and well-differentiated endometrial adenocarcinoma

Nucleous. Electron microscopic findings in the nucleous in the three types of tissue are summarized in Table II. In adenomyotic tissue, the nuclear membrane was distended and smooth in four of five cases. The nuclei were round or oval. Chromatin was more finely distributed in adenomyotic glandular epithelium than in proliferative endometrium or endometrial adenocarcinoma. If the nucleolus was present, there was only one per cell and showed some irregularities.

Therefore one can distinguish the adenomyotic glandular epithelium from the well-differentiated endometrial adenocarcinoma because of the coarse chromatin and the irregularities of the nuclear membrane found in the latter.

Cytoplasm. Electron microscopic findings in the cytoplasm of the three types of tissue are summarized in Table III. In the adenomyotic glandular epithelium, the degree of development of cytoplasmic organelles, such as rough endoplasmic reticulum, free ribosomes, and microfibrillar structure, is between that of proliferative endometrium and well-differentiated endometrial adenocarcinoma. As shown in Table III, some structures seem closer to those of proliferative endometrium, whereas others have more characteristics in common with structures in the well-differentiated endometrial adenocarcinoma.

Comment

I examined the adenomyotic glandular epithelium to study its ultrastructural characteristics and compared them with the electron microscopic findings in normal proliferative endometrial epithelium and those of well-differentiated endometrial adenocarcinoma.

Characteristics of the endometrium ultrastructure in women with a normal cycle are well documented. Ultrastructural differences of the endometrium at different phases of the menstrual cycle are well established; however, ultrastructural diversity of the endometrium in the same phase has also been observed. This diversity depends mainly on the depth of the glands, that is, the depth of the functional and basal layers of the endometrial epithelium. I focused my observations on the surface epithelium inside the lumen and examined the tissue from five patients with different menstrual cycles to ascertain the common characteristics of the heterotopic endometrium when under different hormonal stimulation.

Nilsson[3] reported that few differences exist between normal glandular epithelium and endometrial adenocarcinoma in the early stage. On the other hand, Gompel[4] studied 14 cases of endometrial cancer by electron microscopy and compared those findings with the ultrastructural characteristics of normal glandular epithelium. However, although he found various morphologic changes in endometrial cancer, he could not define those changes as specific for cancer. A year later Thrasher and Richart[5] reported that endometrial adenocarcinoma cells had abnormal nuclei and nucleoli and that the nuclear bodies were similar to those previously described in the neoplasms of other organs. In this study I was able to point to the morphologic characteristics inherent to endometrial adenocarcinoma compared with those of normal endometrium.

In an interesting report Cummins et al.[6] compared endometrioid adenocarcinoma possibly arising in endometriosis with normal endometrium and endometrial adenocarcinoma; they concluded that it was difficult to distinguish between both tissues under electron microscopic examination. In addition, these two tissues had characteristics similar to those of orthotic proliferative endometrium. Kumar and Anderson[7] reported one case of multicentric adenocarcinomatous changes in endometriosis interna and called it malignant adenomyosis. I also demonstrated the presence of adenomyosis intermingled with endometrial cancer in eight patients.[8]

In conclusion, the ultrastructural findings in adenomyotic glandular epithelium reported herein were different from those of proliferative endometrium, and cytoplasmic organelles have some similarities with those of well-differentiated endometrial adenocarcinoma; however, as yet, no proof has been offered that these similarities predispose the adenomyotic endometrium to malignant degeneration. Unlike normal endometrial tissues, the morphologic features of adenomyosis could be related to its invasive properties. Nevertheless, it remains to be clarified whether these features are essential changes of the adenomyotic glandular epithelium or whether they simply reflect morphologic manifestations arising as a result of the ectopic growth of endometrial cells.

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Relationship between the onset of dysmenorrhea and histologic findings in adenomyosis

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An attempt was made to evaluate the relationship between histologic findings of adenomyosis and onset of dysmenorrhea. Thirty-four cases of histologically diagnosed adenomyosis were investigated in this study. One large sample cut along the longitudinal axis was made and examined to evaluate the histologic parameters and CA 125 levels. Dysmenorrhea was classified into grade I (absent or mild) and grade II (severe). Localization of adenomyosis was divided into type I, adenomyosis continuous from the surface endometrium (29 cases), and type II, adenomyosis present on the serous side of the uterus and not continuous from the surface endometrium (five cases). We made type I adenomyosis, which included 13 cases of grade I and 16 cases of grade II, the focus of our investigation. The parameters that correlated with dysmenorrhea were the number of islands of adenomyosis and glands, distance of invasion, relative rate of invasion, and CA 125 levels. The differences between grades I and II were apparent in the frequency distribution tables, with dysmenorrhea occurring at invasion rates of 80% and greater. (AM J OBSTET GYNECOL 1991;165:229-31.)

Key words: Adenomyosis, onset of dysmenorrhea, histologic findings

Uterine adenomyosis is often associated with dysmenorrhea and is usually treated surgically to relieve symptoms. Dysmenorrhea and swelling of the uterus are often pathognomonic of adenomyosis, affecting all layers of the uterus.[1] However, adenomyosis is often associated with uterine myoma[1-3] and is frequently discovered incidentally in the uterus of patients having surgery for uterine myoma as a result of histopathologic examination. Some patients with this condition complain of dysmenorrhea, whereas others have no symptoms. Therefore it is possible to examine the relationship between histologic findings in adenomyosis and the presence of dysmenorrhea by comparing symptoms and histologic findings in these patients.

In the present study the correlation between histologic findings in adenomyosis and the onset of dysmenorrhea was examined.

Material and methods

Thirty-four patients were entered into this study; 9 had adenomyosis only and 25 had adenomyosis associated with uterine myoma. The diagnosis was made on the basis of macroscopic findings obtained during surgery and histologic examination of tissue samples. Patients were between 40 and 59 years old (mean, 46.7 ± 4.5 years).

The uteri excised during surgery were longitudinally incised along the anterior wall and fixed in 10% formalin. Just one large sample cut along the longitudinal axis was stained with hematoxylin and eosin and examined under an optical microscope to examine the following parameters (Fig. 1): (1) localization of adenomyosis—there were two types of adenomyosis: type I (adenomyosis continuous from the surface endometrium) and type II (adenomyosis present on the serosa of the uterus and not continuous from the surface endometrium); (2) thickness of uterine muscle; (3) number of islands of adenomyotic lesions (including glands and stroma); (4) number of glands; (5) mean number of glands contained in one island of adenomyosis; (6) distance between surface endometrium and the deepest portion of islands in the invaded tissue; (7) ratio of the depth of adenomyosis to uterine muscle thickness; (8) status of adenomyotic lesions, that is, lesions were classified as being in the proliferative or secretory phase; (9) presence or absence of hemorrhage in adenomyotic lesions; and (10) preoperative CA 125 levels.

Dysmenorrhea was classified into the following two categories: grade I (dysmenorrhea is absent or slight) and grade II (marked dysmenorrhea is present or analgesics are required during menstruation).

Results

Histologic findings and CA 125 levels

Localization of adenomyosis uteri. Types I and II were found in 29 and 5 cases, respectively. Patient ages, number of islands of adenomyosis and glands, dysmenorrhea, and CA 125 blood levels in the type of adenomyosis are shown in Table I. There were significant differences between the two types in the number of islands and glands and CA 125 levels in the blood. The incidence of dysmenorrhea was significantly lower in

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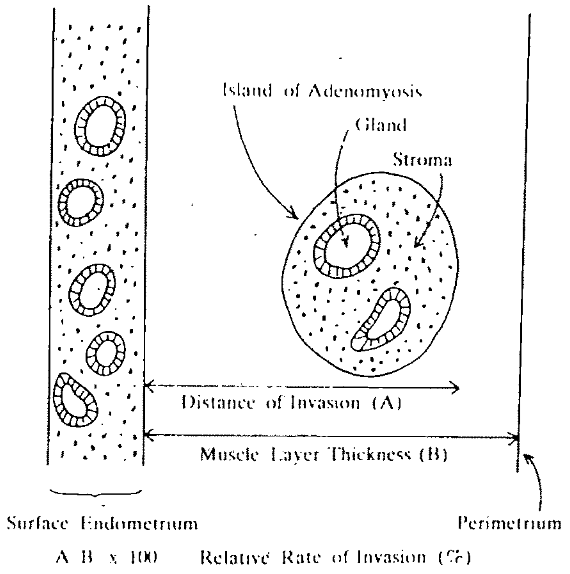

Fig. 1. Diagram of location of adenomyosis in the myometrium.

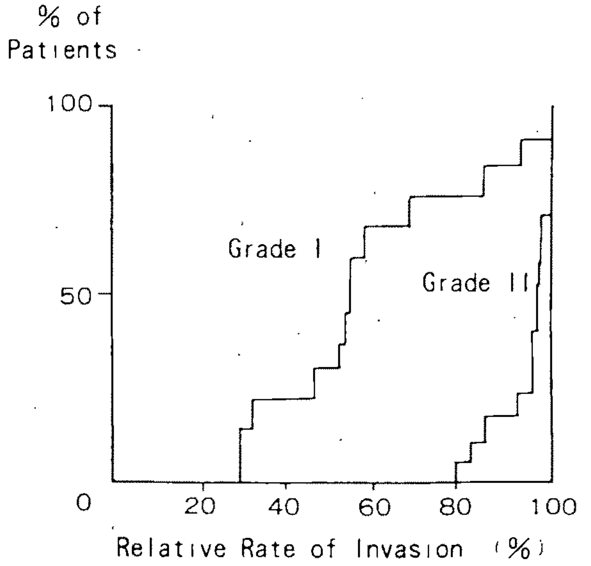

Fig. 2. Relative cumulative frequency distribution of patients.

Table I. Outcome data for localization of adenomyosis

Data	*Type I* (*n* = 29)	*Type II* (*n* = 5)
No. of islands	134.7 ± 122.0	15.2 ± 16.6
No. of glands	623.1 ± 657	99.8 ± 124.9
Dysmenorrhea		
Grade I (No.)	13	4
Grade II (No.)	16	1
CA 125 value (U/ml)	109.4 ± 137.9 (5.2-579.0)*	30.2 ± 30.8 (6.0-84.0)*
Age (yr)	46.9 ± 4.7	45.8 ± 2.4

Data are mean ± SD.
*Lower and upper values.

type II. There were no significant differences in patient ages. Only the 29 patients with type I adenomyosis were evaluated in the other parameters (Table II).

Muscle layer thickness. Uterine muscle layer thickness was comparable between grade I (42.1 ± 20.2 mm [mean ± SD]) and grade II (45.0 ± 15.4 mm).

Number of islands of adenomyosis. The total number of islands per section of uterine muscle layer was significantly greater for grade II (197.7 ± 125.9) than for grade I (58.6 ± 60.3) ($p < 0.01$).

Number of glands. The number of glands per section of uterine muscle was significantly greater for grade II (840.4 ± 629.2) than for grade I (255.6 ± 612.4) ($p < 0.05$).

Mean number of glands per island of adenomyosis. The mean number of glands per island of adenomyosis was comparable for grades I (6.3 ± 6.8) and II (5.1 ± 4.6).

Distance between the surface endometrium and the deepest portion of the adenomyotic lesion. The distance between the surface endometrium and the deepest portion of the adenomyotic lesion was significantly greater for grade I (42.9 ± 16.1 mm) than for grade II (24.9 ± 16.6 mm) ($p < 0.01$).

Ratio of the depth of adenomyosis to uterine muscle thickness. The degree of invasion estimated based on the depth/muscle layer thickness × 100 (percent) was significantly greater for grade II (94.3% ± 6.7%) than for grade I (57.9% ± 23.3%) ($p < 0.01$).

Status of adenomyotic lesions. There were no significant differences between grades I and II.

Presence or absence of hemorrhage from adenomyotic lesions. There were no significant differences between grade I and II.

CA 125 levels. Preoperative CA 125 levels in the blood were significantly higher for grade II (162.2 ± 161.0 U/ml; range, 6 to 579 U/ml) than for grade I (37.9 ± 44.9 U/ml; range, 5.2 to 60 U/ml) ($p < 0.02$).

Frequency distribution analyses. Frequency distribution tables were prepared with respect to the items that showed significant differences between the two groups to determine the borderline at which grade I dysmenorrhea progresses to grade II dysmenorrhea.

Number of islands of adenomyotic lesions. All eight patients with 200 or more islands of adenomyotic lesions were in grade II. However, 6 of 16 patients (37.5%) with less than 50 adenomyotic lesions were also in grade II, and no clear borderline could be determined.

Number of glands. Ten of the 13 patients with 500 or more glands were in grade II. However, no clear borderline could be determined because seven patients with less than 500 glands were also in grade II.

Distance between the surface endometrium and the deepest portion of the adenomyotic lesion. Five of the six patients with distances between the surface endometrium and the deepest portion of the adenomyotic lesion of 50 mm and over were in grade II. However, the borderline

Table II. Outcome data for grade of dysmenorrhea

Data	*Grade I* *(n = 13)*	*Grade II* *(n = 16)*	*p*
Muscle layer thickness (mm)	42.1 ± 20.2	45.0 ± 15.4	NS
No. of islands	58.6 ± 60.3	197.9 ± 125.9	<0.01
No. of glands	255.6 ± 612.4	840.4 ± 629.2	<0.05
Mean No. of glands	6.3 ± 6.8	5.1 ± 4.6	NS
Depth of invasion (mm)	24.9 ± 16.6	42.9 ± 16.1	<0.01
Relative rate of invasion (%)	57.9 ± 23.3	94.3 ± 67.0	<0.01
CA 125 (U/ml)	37.9 ± 44.9 (5.2-60.0)*	162.2 ± 161.0 (6.0-579.0)*	<0.02

Data are mean ± SD.
*Lower and upper values.

between grades I and II could not be drawn based on absolute glandular depth.

Ratio of the depth of adenomyosis to muscle layer thickness. The frequency distribution table prepared regarding the degree of glandular invasion showed that all 19 patients with invasion rates of 80% and over were grade II. A relative cumulative frequency distribution table was then prepared (Fig. 2). Differences between grades I and II were apparent in this table, with dysmenorrhea occurring at invasion rates of 80% and greater.

Comment

Since the report by Cullen,[4] the histogenesis of uterine adenomyosis has been explained as a deep proliferation of the normal endometrium. It is well-known that uterine adenomyosis causes dysmenorrhea and occurs mainly in multiparous women,[1,3,5] in particular in those aged 35 years and over.[2,5,6] However, adenomyosis is often associated with uterine myoma and is often discovered incidentally in patients undergoing surgery for uterine myoma.[1-3] Dysmenorrhea may not necessarily be present in these patients, and it may be absent despite the presence of adenomyosis. Therefore it is likely that dysmenorrhea occurs when adenomyosis has progressed to some extent.

In the present study we determined the relationship between histologic findings in adenomyosis and the onset of dysmenorrhea based on histologic examination of adenomyosis.

The 29 patients with histologic evidence of adenomyosis, 16 with dysmenorrhea and 13 without dysmenorrhea, were compared. Significant differences were observed between the two groups in the number of islands of adenomyosis, number of glands, distance between the surface endometrium and deepest adenomyotic lesion, and the ratio of the depth of adenomyosis to uterine muscle thickness. There were no significant differences between the two groups in muscle layer thickness, number of glands per island of adenomyosis, status of adenomyosis, or the presence or absence of hemorrhage in adenomyosis. CA 125 levels were correlated with the severity of dysmenorrhea.

Frequency distribution tables were prepared for the items that showed close correlation with the severity of dysmenorrhea to determine the borderline at which dysmenorrhea occurs. No clear borderline was observed with respect to the number of islands of adenomyotic lesions and glands or depth of adenomyosis. However, a clear borderline was found regarding the extent of glandular invasion, with dysmenorrhea occurring when glandular invasion exceeds 80% or more of the myometrium. This means, for example, that dysmenorrhea occurs when the depth of invasion exceeds 1.6 cm in patients with a 2 cm thick muscle layer. In patients with a 5 cm thick muscle layer, symptoms occur when the depth of invasion exceeds 4 cm. This finding suggests that dysmenorrhea is not only caused by increases in the amount or size of adenomyosis in the uterine muscle layer but also by the extent of muscle invasion.

In this study population, the incidence of dysmenorrhea was not high in patients with adenomyotic lesions only on the serous side, which suggests that invasion up to the perimetrium is not the only requirement for the onset of dysmenorrhea. Dysmenorrhea probably occurs when the volume of adenomyotic lesions as a percentage of the total uterine volume exceeds a certain threshold. Dysmenorrhea appears to be the result of tonic stimulation of the entire uterus by adenomyosis. This stimulation seems to be induced when the depth of invasion exceeds 80% of the total muscle layer.

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Animal model of uterine adenomyosis: Is prolactin a potent inducer of adenomyosis in mice?

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Uterine adenomyosis is a benign lesion but a serious disease for women, because the symptoms are characterized by abnormal bleeding, pain, cramps, and sterility. Thus elucidation of the mechanisms involved in the development of the disease would contribute to improved management and treatment and prophylaxis of this lesion. A mouse model, in which ectopic pituitary isografting is associated with an increase in the plasma level of prolactin, is useful for a rapid and frequent induction of uterine adenomyosis and can be used to study the origin of this lesion. (Am J Obstet Gynecol 1991;165:232-4.)

Key words: Adenomyosis, mouse, animal model, prolactin

Uterine adenomyosis is a benign pathologic disorder characterized by ectopic growth of the endometrial tissues, stroma, or glands or both into and beyond the myometrium. Spontaneous development of adenomyosis with advanced age has been reported in several species of animals, including nonhuman primates.[1-6] Many research reports deal with the development of uterine lesions, but almost all have concentrated on the development of uterine cancer. However, uterine adenomyosis, which is considered one of the causes of abnormally prolonged and profuse bleeding, cramps, and sterility in humans and animals,[3, 7] has not attracted much attention. Presently the endocrine mechanisms responsible for the genesis of adenomyosis are not well understood. Therefore it would be of great value to establish an animal model for uterine adenomyosis to study the mechanisms involved in its development; a better understanding of the origin and pathophysiology of adenomyosis will contribute to improved management and treatment, as well as prophylaxis, of this lesion in humans.

We have found that ectopic pituitary transplantation results in an early development of adenomyosis and at a high incidence in mice; in mice adenomyosis was associated with an elevated plasma level of prolactin.[8-12] These findings led us to believe that in mice chronic hyperprolactinemia is a major hormonal factor in the genesis of adenomyosis. This article reviews experimental methods for induction of adenomyosis and discusses the mechanisms of the development of this human disease based on data obtained from animal models.

Experimentally induced adenomyosis

The most commonly used method for the induction of adenomyosis is long-term estrogen therapy (usually more than 6 months). A pioneering report on the development of adenomyosis after long-term estrogen treatment was published in 1935[13]; mice were treated with estrogen for more than 8 months. After this finding, prolonged treatment with estrogen was established as an effective method for the induction of adenomyosis in mice,[14, 15] guinea pigs,[16, 17] rabbits,[18] and monkeys.[19, 20] It is well known that normal and abnormal proliferation of uterine tissues are mainly under the control of ovarian steroid hormones (estrogen and progesterone). Furthermore, Ostrander et al.[21] found a high incidence of adenomyosis in newborn mice treated with diethylstilbestrol in combination with progesterone and also in adult mice treated with progesterone plus estrogen. However, subcutaneous implantation of progesterone alone also enhanced the development of adenomyosis in mice,[21-23] suggesting that a high level of circulating progesterone is also a favorable factor for the genesis of this lesion.

Nevertheless, we recently reported that treatment with prolactin during the neonatal period (1 to 14 days of age) or young adult life (40 to 79 days of age) enhanced the development of adenomyosis in mice.[24] In addition, the administration of dopamine antagonists, which are potent enhancers of pituitary prolactin release, resulted in a high incidence of adenomyosis in mice (Singtripop et al. Unpublished data). These results strongly suggest that prolactin plays a key role in the development of uterine adenomyosis.

Moreover, prenatal exposure of mice to diethylstilbestrol resulted in a higher incidence of adenomyosis at advanced ages.[25-27] Prenatal and neonatal expo-

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sure to estrogen or diethylstilbestrol results in a hormonal imbalance because of the altered function of the hypothalamo-hypophysial-ovarian axis,[28, 29] which may contribute to the development of adenomyosis. In contrast, it has been reported that similar neonatal treatment of mice with estrogen failed to induce adenomyosis, whereas in untreated control mice adenomyosis developed at an advanced age.[30] It is well known that decreased responsiveness to the growth-promoting effect occurs in the uterus of mice treated perinatally with estrogen.[28, 29] Thus it is also plausible that a decreased sensitivity of the uterus to estrogen could result in a low incidence of adenomyosis.

We have found that in mice an ectopic isograft of an anterior pituitary gland into the uterus induces adenomyosis at an early stage and at a high incidence; in this case adenomyosis was always associated with hyperprolactinemia.[8-12, 31] In these studies adenomyosis could be induced within a month after grafting although there were some strain differences regarding the latency period of development.[32] Huseby et al.[33, 34] confirmed our findings regarding the effect of pituitary grafting in mice and reported a significant rise in the serum level of progesterone in addition to that of prolactin. These findings imply that prolactin may play an important role in the development of adenomyosis, although a synergistic effect of progesterone and estrogen with prolactin cannot be excluded.

Histogenesis of adenomyosis in mice

This new method for the induction of adenomyosis by pituitary grafting[8-12] makes it possible to examine the histogenesis of adenomyosis because of the extremely short latency period between induction and development of the lesion. The earliest observable histologic change in the uterus is the infiltration of endometrial stromal cells into the inner myometrium, along the blood capillaries or lymphatic vessels. After invasion by a large number of stromal cells, uterine glands track the routes of the stromal cells and translocate into the musculature. The aberrant endometrial tissues then reach the connective tissue space between the inner and outer smooth muscle layers. Associated with these changes, the inner smooth muscle bundles of the myometrium become loosened and thinner.

Electron microscopy has revealed that noticeable changes in the morphology and arrangement of smooth muscle cells take place.[35, 36] The smooth muscle cells of the myometrium become markedly loosened unlike the muscle cells running concentrically around the uterine axis in normal control mice. In addition, muscle cells become irregular in shape and are markedly reduced in size, which result in wider intercellular spaces than those in normal control mice. Disintegration of the muscle layer, scantiness of cell organelles, and pyknosis of muscle cells are frequently observed in adenomyotic areas. These findings indicate that the first step, invasion of the myometrium by endometrial tissues, is caused by the degeneration of the muscle layer and that in the normal state the muscle layer protects tissues from invasion by proliferating endometrial tissue into the musculature.[35, 36]

Causes of the development of adenomyosis

As previously mentioned, we found that in mice ectopic isograft of the anterior pituitary gland induces adenomyosis at an early stage and at a high incidence; moreover, the development of adenomyosis was always associated with hyperprolactinemia.[11, 12] Furthermore, the spontaneous development of adenomyosis with advanced age could be prevented by the administration of bromocriptine, a potent suppressor of pituitary prolactin release, which was administered only for 4 weeks beginning at 4 weeks of age.[37, 38] In addition, the administration of prolactin during young adult life resulted in a high incidence of adenomyosis,[24] and the administration of dopamine antagonists, which are potent inducers of pituitary prolactin release, also enhanced the development of adenomyosis. All these findings are in agreement with our hypothesis that prolactin is the primary hormone involved in the development of adenomyosis in mice.

It is evident that prolonged treatment with estrogen results in a high incidence of adenomyosis. Since estrogen is a potent stimulant of pituitary prolactin secretion, long-term treatment with estrogen implies a possible participation of prolactin in the genesis of adenomyosis. In this respect, Ostrander et al.[21] claimed that the effect of prolonged treatment with progesterone, alone or in combination with estrogen, was not indicative of any role of prolactin in the genesis of adenomyosis, because progesterone prevented estrogen-induced prolactin elevation in rats. However, it is well known that prolactin stimulates progesterone secretion from the ovary, and it further enhances the effect of progesterone on the target cells,[39] perhaps through the modulation of progesterone receptor activity.

On the contrary, it has been reported that prolactin exerts a marked inhibitory effect on oxytocin-induced myometrial contraction both in rats primed with estrogen and progesterone[40] and in pregnant rabbits.[41] These findings indicate a close relationship between prolactin and function of the myometrium and suggest that prolonged exposure of the musculature to prolactin might cause myometrial degeneration as observed by electron microscopy.[35, 36] The degeneration of the myometrium may be responsible for the invasion by endometrial tissue into it; prolactin directly affects the myometrium and leads to the degeneration of muscle cells. In conclusion our mouse model of uterine adenomyosis strongly suggests that prolactin is a trigger in the genesis of adenomyosis and that estrogen and progesterone facilitate the development of the lesion as promoters.

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LETTERS TO THE EDITORS

The Cherney incision for gynecologic cancer

To the Editors: The excellent article on the Maylard incision (Helmkamp BF, Krebs H-B. The Maylard incision in gynecologic surgery. AM J OBSTET GYNECOL 1990;163:1554-7) confirms the value of transverse incisions for gynecologic cancer surgery. I wonder whether the authors have experience with the use of the Cherney incision?

The Cherney incision involves detachment of the rectus abdominus tendon near its insertion on the pubic symphysis. Compared with the Maylard incision, it is much faster to perform, an average of 1 to 2 minutes versus 5 to 10 minutes. No drains are required because there is virtually no bleeding from the tendon, thus the risk of hematoma and seroma are markedly reduced as compared with the Maylard incision. In addition, it is often possible to spare the inferior epigastric vessels, maintaining vascularity to the rectus muscles. The major disadvantage of the Cherney incision is that occasionally there is poor development of the tendon sheath, which contraindicates this incision. In addition, about 1% to 2% of patients may rupture the tendon postoperatively if it is not repaired carefully or if there is excessive postoperative tension. Nonetheless, the Cherney incision offers greater abdominal wall strength because the rectus muscles are not required to heal by fibrosis as with the Maylard incision. We have found this to be an excellent incision for radical hysterectomy and would be interested in the authors' comments. Finally, the authors refer to the Maylard incision as a "muscle-splitting" incision (e.g., McBurney) when "muscle cutting" would be more accurate.

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Reply

To the Editors: I appreciate the comments regarding our article on the Maylard incision. The Cherney incision[1] was introduced in 1941 as a variation of other transverse incisions and the pros and cons of these various incisions are discussed at length by Christopherson in his excellent article.[2] I have no personal experience with this incision; I use the Maylard incision exclusively when optimal pelvic exposure is required.

I would agree with you that "muscle cutting" would more accurately describe the Maylard technique, inasmuch as the incision through the rectus muscles is perpendicular to the muscle fibers rather than parallel.

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Costs and benefits of examining all placentas

To the Editors: I read with interest the article by Salafia and Vintzileos (Salafia CM, Vintzileos AM. Why all placentas should be examined by a pathologist in 1990. AM J OBSTET GYNECOL 1990;163:1282-93) in which they advocate examination of all placentas by pathologists. In calculating the cost-effectiveness of such examination, the authors made the following assumptions that I believe are faulty:

1. An average obstetrician will be successfully sued for damages of $5 million to $6 million over his or her career.

2. Examination of placentas will clear the obstetrician of malpractice charges in all such cases in which he/she is sued.

Further comments from the authors on the above assumptions would be helpful to estimate the true cost-effectiveness of examining all placentas.

Khush Mittal, MD

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Reply

To the Editors: We thank Dr. Mittal for his comments. We do not, however, state that the average obstetrician will be successfully sued for $5 million to $6 million, but that in many states the average "award" for medical negligence in cases of cerebral palsy is $5 million to $6 million.

We also do not state, and certainly do not believe, that examination of the placenta will clear the obstetrician of charges in all such cases in which he/she is sued. We state that given the cost per award, the generated cost (from placental examination) is worthwhile to avoid *one* case of alleged medical malpractice. In our experience, subclinical placental pathology is frequently present in cases brought to litigation and may provide data supportive of the defense.

We appreciate the opportunity to clarify these points.

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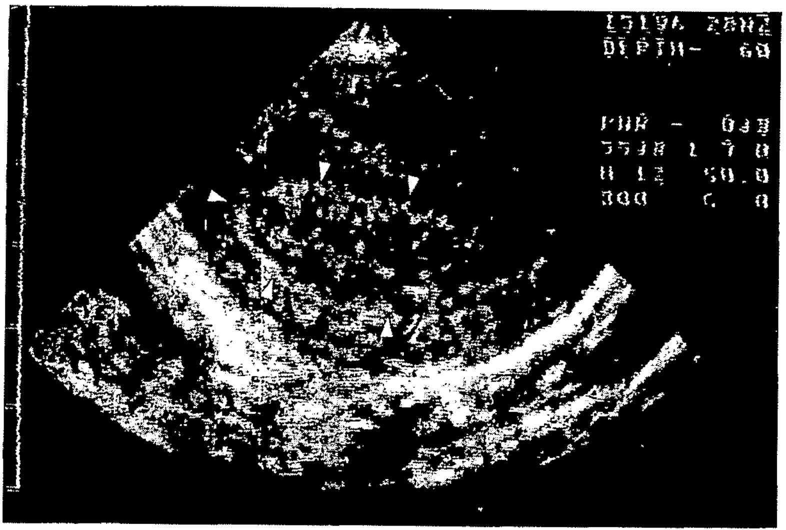

Fig. 1. This cross section of the uterus in a patient with an ectopic pregnancy shows a thick prominent decidua (outlined by *arrowheads*) that contains a few small vascular structures (2 mm anechoic areas).

Ultrasonographic visualization of endometrial vascularity with ectopic pregnancy

To the Editors: We wish to report to you and your readers that some patients with ectopic pregnancy may demonstrate endometrial vascularity on ultrasonographic examination. This is associated with a thick, prominent endometrium without other intrauterine findings of pregnancy.*

Most patients had widespread findings in the inner portion of the endometrium (decidua), which was generally hypoechoic compared with the more hyperechoic outer portion of the endometrium.[1] Sometimes we were able to show discrete vascular channels up to 2 mm in diameter (Fig. 1) with rapid or tumbling flow, whereas in other patients the flow was less well contained and appeared as scattered pulsatile-like foci or sluggish flow without discrete identifiable vessels. We propose that these observations are a result of vascular alterations known to occur in the decidua of pregnant patients. We believe these findings are most likely a result of venous structures, although it is possible that some of the pulsatile-like findings are arterial in origin.

This finding of endometrial vascularity is not specific for ectopic pregnancy. We have seen this, on occasion, with incomplete abortions with microscopic villi, complete abortions with retained decidua, in one intrauterine pregnancy, before visualization of the gestational sac and, probably pulsatile-like flow (without discrete vessels) in one nongravid patient.

Of interest, a cross section or oblique section of a vessel(s) may produce one or more hypoechoic or anechoic areas within the endometrium. This could be confused with an early intrauterine pregnancy, an abnormal intrauterine pregnancy, an early molar pregnancy, a pseudogestational sac, an endometrial cyst, a polyp with cystic degeneration, cystic endometrial hyperplasia, or less common types of endometrial pathology. This pitfall may be avoided if blood flow is seen through the vessel(s) or if a more ideal scan plan shows a clearly defined vascular structure(s).

Many questions remain unanswered (e.g., Is the visualization of endometrial vessels specific for pregnancy?) and further research needs to be performed. We would be interested in hearing the findings other investigators have in this regard.

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*An Acuson 128 ultrasonography machine with a 5 MHz vaginal probe with gray-scale technique and 0 persistence was used. At times we had the mother hold her breath for several seconds to eliminate breathing artifacts.

High blood pressure in pregnancy

To the Editors: We believe that the National High Blood Pressure Education Program Working Group report on high blood pressure in pregnancy (AM J OBSTET GYNECOL 1990;163:1691-712) obscures an important issue through two contradictory statements. The section "Rationale for treatment" contains this advice: "... the cornerstone of obstetric management of preeclampsia is based on a decision as to whether the infant is more likely to survive in utero or in the nursery."

Relevant studies have long indicated that neonatal survival rates peak at the time of the expected date of confinement and decline as little as 1 week thereafter.[1] Accordingly, the philosophy of effecting delivery by 40 weeks' gestation both in acute and chronic hypertensive disorders has prevailed for decades in obstetric textbooks.[2,3] Because with contemporary management no tangible improvement in neonatal mortality and morbidity rates can be expected beyond week 38, the above statement encourages termination of pregnancy at "term" and certainly no later than the "expected date of confinement." However, the section "Fetal indications" pronounces a different recommendation: ". . . if the maternal condition is stable, delivery is indicated by signs of abnormal fetal function. If fetal growth and well-being remain normal, pregnancy should proceed to spontaneous labor."

Spontaneous labor normally occurs between 38 and 42 weeks' gestation. Only thereafter does the threat of postmaturity warrant intervention. Accordingly, the last mentioned recommendation invites waiting for labor until week 42.

Over our objections, some physicians in this geographic area embraced the last quoted management pattern in recent years and refrained from intervention in "mild" preeclampsia until week 42. This endeavor repeatedly entailed unfortunate fetal and even maternal outcomes. Consequently, whereas "severe" cases after 28 weeks' gestation almost invariably ended favorably, painful losses have resulted from conservatively treated "mild" preeclampsia.

We are familiar with the writings of various members of the Working Group and perceive the earlier cited inconsistent viewpoints as an unsuccessful attempt at reconciling two incompatible approaches to management. As a result, the report, in our opinion, fails to clarify the appropriate treatment for mild preeclampsia. With regard to its impact on perinatal statistics, this omission may offset the benefits of this prodigious work insofar as mild cases occur far more often than do severe ones. In terms of absolute numbers, we probably encountered more catastrophes from mild than from severe preeclampsia in the recent past. We fear therefore that a narrow interpretation by obstetricians of the instruction in "Fetal indications" section may lead to a similar experience nationwide.

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Maternal hemodynamics in pregnancy

To the Editors: Ikeda and Mori (Ikeda T, Mori N. Assessment of cerebral hemodynamics in pregnant women by internal carotid artery pulsed Doppler velocimetry. AM J OBSTET GYNECOL 1990;163:494-8) state that to date no studies have been done of serial changes in maternal cerebral hemodynamics during pregnancy. Obviously the authors were not aware that we have examined the carotid artery, femoral artery and vein, and uterine vessels serially and longitudinally during pregnancy by Doppler ultrasonography.[1] For the carotid artery we also noticed a significant decrease of peak systolic and end-diastolic flow velocities as well as a decrease in mean velocity that was, however, not significant.

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Reply

To the Editors: Drs. Baumann and Huch measured velocity in the common carotid artery. This artery divides into internal and external carotid arteries. The external carotid artery supplies blood to extracranial organs and the internal carotid artery feeds the brain. Blood flow velocity waveforms in the common carotid artery combine a high-resistant flow in the external and a low-resistant flow in the internal carotid artery.[1] When cerebral blood flow assessed by xenon 133 inhalation technique was compared with the velocity in the carotid artery measured with Doppler technique, the correlation coefficient between mean hemispheric cerebral blood flow and internal carotid end-diastolic velocity was 0.83 in the left side and 0.88 in the right side; that of common carotid artery velocity and mean hemispheric cerebral blood flow was 0.69 in the left side and 0.71 in the right side.[2] It is apparent that common carotid artery velocimetry is less specific in the assessment of cerebral blood flow.

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Epidemiology of preeclampsia and eclampsia

To the Editors: We read the article by Saftlas et al. (Saftlas AF, Olson DR, Franks AL, Atrash HK, Pokras R. Epidemiology of preeclampsia and eclampsia in the United States, 1979-1986. AM J OBSTET GYNECOL 1990;163:460-5) with great interest and share the concern about the apparent steady trend of increasing rates between 1983 and 1986. Whereas diagnosis-related groups may indeed have prompted more accurate reporting and even overdiagnosing, we have found that reliance on discharge diagnoses at our institution with the use of *International Classification of Diseases, Injuries, and Causes of Death,* 9th revision (ICD 9) categories, as was done in this study, is enormously problematic for studies of hypertensive disorders in pregnancy.[1] First, fully 25% of the cases generated by ICD 9 codes at our institution proved to be incorrect diagnoses and were excluded after review of the hospital records. Second, review of all hospital records documented that 53.3% of all true cases had been missed by the coders at our insitution.

Given this, we wonder what Saftlas et al. may have done to verify the diagnoses in the National Hospital Discharge Survey data base and to check on the completeness of ascertainment. Clearly this presents a formidable task, but it is nevertheless crucial. Our data suggest that the national rates of preeclamspia and eclampsia may well be at least twice that reported in this article.

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REFERENCE

1. Ales KL, Charlson ME. In search of the true inception cohort. J Chron Dis 1987;40:881-5.

Reply

To the Editors: We thank Drs. Ales and Charlson for their interest in our article. We are not suprised that the national rate of preeclampsia (2.6%) is lower than that found at individual hospitals such as The New York Hospital because such hospitals serve as major referral centers for high-risk pregnancies. Moreover, because of the demographic makeup of the obstetric population at The New York Hospital, the rates of obstetric conditions in this population almost certainly differ from the rates observed in the general obstetric population of the United States.

We agree with Drs. Ales and Charlson that the sensitivity and specificity of discharge diagnoses are not perfect. We were unable, however, to review the medical records included in our study; to preserve the confidentiality of the participating hospitals, the National Hospital Discharge Survey does not permit outside reviews. We have, however, had the opportunity to verify discharge diagnoses of preeclampsia from a population-based study we are conducting among Navajo Indian women. When we reviewed medical records from the five hospitals in the study, we found that we had underestimated the incidence by 25% when we had used hospital discharge diagnoses as our data source.

In the validation study by Ales and Charlson,[1] the accuracy of all hypertension complicating pregnancy was assessed, not just preeclampsia. Clearly, the validity of hospital discharge diagnoses can be expected to vary depending on the disease conditions under study.[2] Our review of their article indicates that at their institution, the rate of all hypertension complicating pregnancy based on hospital discharge diagnoses (7.5%) was underestimated by 57%. Thus it is not clear to us why Drs. Ales and Charlson believe that the national rate of preeclampsia was underestimated at least 100% when their data do not indicate this. Even if the national rate of preeclampsia had been underestimated by as much as 57%, the adjusted rate would be only 4.1%, a rate much lower than those reported by most hospital-based studies.

The rates of preeclampsia and eclampsia estimated from the National Hospital Discharge Survey are the only nationally representative rates published to date. Although these may underestimate the true incidence, we do not believe that the underestimate is as high as 100%. These data can be used to track trends over time and compare rates across geographic areas, but because they are population-based they should not be expected to compare to rates derived from large referral medical centers.

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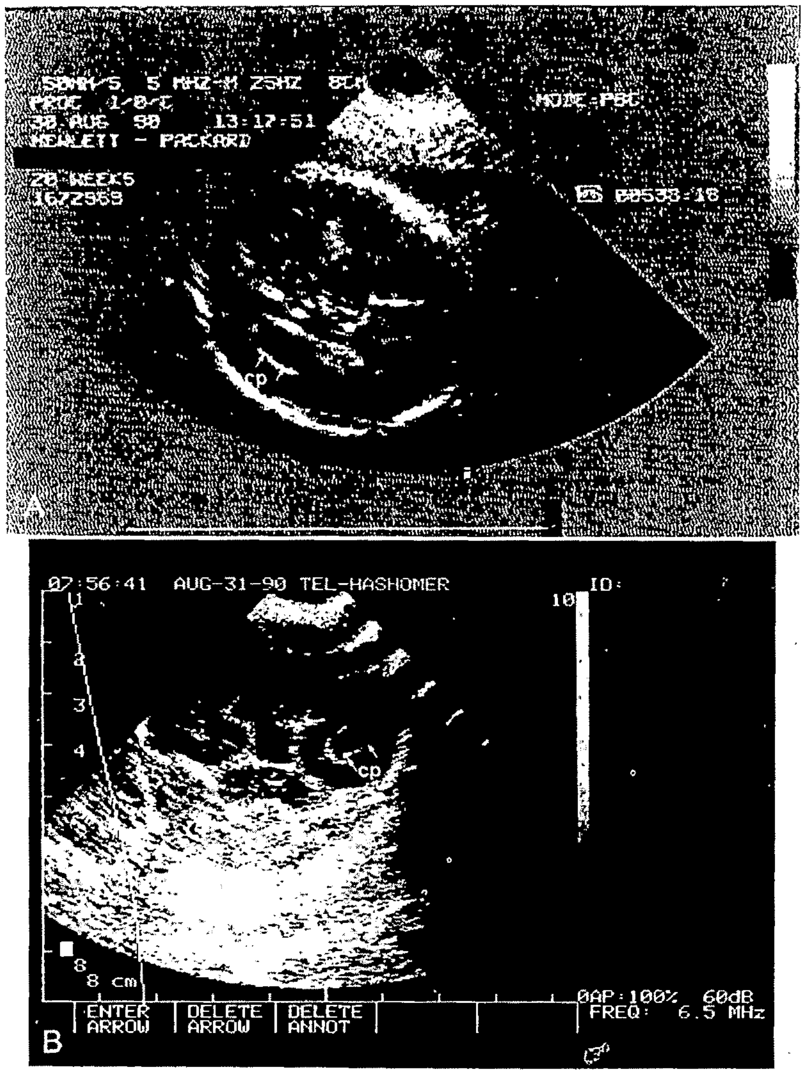

Fig. 1. A, Axial in utero scan shows small choroid plexus cyst at 19 weeks' gestation in a fetus with trisomy 18. **B,** Postabortion frontal scan (6.5 MHz) shows the same ultrasonographic appearance as in utero.

Are choroid plexus cysts an indication for second-trimester amniocentesis?

To the Editors: Drs. Benacerraf, Harlow, and Frigoletto evaluated the risk of trisomy 18 in fetuses with choroid plexus cysts of the lateral ventricle (Benacerraf BR, Harlow B, Frigoletto FD. Are choroid plexus cysts an indication for second-trimester amniocentesis? AM J OBSTET GYNECOL 1990;162:1001-6). The authors used statistical analysis to hypothesize that the risk of trisomy 18 is one for every 477 normal fetuses with choroid plexus cysts and their recommendation was to avoid amniocentesis, which has a risk of 1:200. We would like to argue with these findings and conclusions.

To evaluate the significance of choroid plexus cysts in the general population, the authors averaged the prevalence of choroid plexus cysts found in four studies (0.18%, 0.65%, 0.80%, 2.5%[1-4]), which resulted in 1%, an estimation found to be in agreement with their experience. However, the highest rate of 2.5% is not a representative figure because the study by Chan et al.[4] was performed on a small series of 513 women at level II ultrasonographic examination, resulting in a biased overestimated rate. Therefore this rate should be omitted from calculation. A large prospective study performed by us on 5400 low-risk women revealed a 0.6% rate of fetal choroid plexus cysts.[5] The addition of these 5400 cases to the other four large studies[1-3, 6] results in a total of 28,292 fetuses screened at midtrimester with an average 0.7% prevalence of choroid plexus cysts. Therefore it seems more appropriate to use 0.7% rather than the estimated 1% rate.

Furthermore, in their analysis the authors reviewed

26 fetuses with trisomy 18 and found 30% choroid plexus cysts in these cases. These 26 cases were divided into two groups: 13 fetuses with trisomy 18 who were first seen for amniocentesis (group I, Table I) and 13 fetuses with trisomy 18 who did not present with amniocentesis but were referred to them (group II, Table II). The prevalence of choroid plexus cysts in the first group was 5/13 (38%), whereas the prevalence in the second group was one of eight cases at midtrimester (12%), which has a significant statistical difference ($\chi^2 = 4.5$; $p < 0.01$). This is surprising because we would expect that in the referral patients, which is a selected group, there would be more affected cases. A possible explanation for this difference might be the sonographers' unawareness of the problematic issue of fetal choroid plexus cysts because the first publication on this subject by Chudlight et al.[6] appeared in 1984, whereas their study commenced on Jan. 1, 1983.

Moreover the 30% prevalence of choroid plexus cysts in trisomy 18 found by Benacerraf et al. seems lower in comparison with the interesting ultrasonographic study performed by Fitzsimmons et al.[7] They found among 14 aborted fetuses with trisomy 18, 71% (5/7) had choroid plexus cysts at midtrimester. Benacerraf et al. claimed that the high rate obtained by Fitzsimmons et al. was a result of the in vitro study, which enabled better resolution of the lesions. We do not agree with this speculation because we compared the in vivo and in vitro appearance of choroid plexus cysts and obtained the same size and lesion shape prenatally and postnatally (Fig. 1, *A* and *B*).

Although it is clear that choroid plexus cysts can occur in fetuses with trisomy 18, the risk of such a chromosomal abnormality in a fetus with an incidental finding of choroid plexus cysts is difficult to determine. Because trisomy 18 is an uncommon disease that occurs three per 10,000 births, a large prospective study is needed to resolve this problem. While awaiting such a definitive study, the question of how to manage such pregnancies remains. Indirect estimation on the basis of published data may be of value. We reviewed the literature and collected data only on fetuses at midtrimester (15 to 24 weeks' gestation) with trisomy 18 and choroid plexus cysts; sporadic case reports were excluded. A total of 33 cases with midtrimester diagnosis of trisomy 18 were cumulated; among them were 18 (55%) fetuses with choroid plexus cysts. Thus we can conclude that the known prevalence of choroid plexus cysts in trisomy 18 is about 55%.

With these figures in mind we can proceed with the authors' hypothesis that for 33 cases with trisomy 18 a hypothetical sample of 110,000 women is needed. With a 0.7% prevalence of choroid plexus cysts, 770 normal cases with this abnormality would be detected. It is known that 30% of these cases have no other detectable anomalies on ultrasonography. Multiplication of 30% per 18 cases results in a product of 5.4 fetuses with trisomy 18 with choroid plexus cysts and without other findings, or one fetus with trisomy 18 for every 142 (770/5.4) normal fetuses with choroid plexus cysts. This is higher than a 35-year-old woman's risk of having a child with trisomy 21, which is 0.27%, a widely accepted threshold for consideration of amniocentesis. We therefore suggest that until a larger prospective study determines the real risk of trisomy 18 in the fetus with choroid plexus cysts, amniocentesis is warranted.

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Reply

To the Editors: We appreciate the interest in our work shown by this letter; however, we do not agree with the conclusions reached concerning either the prevalence of choroid plexus cysts in the general population or the prevalence of choroid plexus cysts in fetuses with trisomy 18. We averaged the prevalence of choroid plexus cysts in the four studies cited because these were indeed the largest studies of their kind available. I see no reason to omit either the highest or the lowest prevalence among these four and certainly cannot include the authors' series, which has yet to be published in a peer-reviewed journal. Therefore in our opinion the best estimate of choroid plexus cysts in the general fetal population in the mid phase of the second trimester is 1%, which seems to be in keeping with our experience.

The authors of the letter question the prevalence of choroid plexus cysts in the first group versus the second group of fetuses with trisomy 18 in our article. The difference in prevalence may be a result of the second group being strictly referrals for fetal anomalies, whereas patients in the first group were undergoing amniocentesis for advanced maternal age and therefore were a different subpopulation. The prevalence of choroid plexus cysts suggested by Fitzsimmons et al., however, is still thought to be an overestimate because they used higher frequency transducers, which are known to have better resolution than those used for in vivo obstetric ultrasonography. Even though the authors of the letter show one case of a large choroid plexus cyst seen both in vitro and in vivo, this does not in any way demonstrate the difference in the identification of smaller choroid plexus cysts that may not be quite as obvious. The prevalence of a small structure seen in vitro with higher resolution transducers cannot be applied to an in vivo experience unless a proper control study is included.

Furthermore, the authors of the letter have come up with a prevalence of choroid plexus cysts of 55% among fetuses with trisomy 18. There is absolutely no basis for this number because they have gathered cases of trisomy 18 at random in the literature, most of which are being reported because of the presence of choroid plexus cysts. Therefore the denominator of all the fetuses with trisomy 18 coming from all these different laboratories is totally unknown and the prevalence of choroid plexus cysts among fetuses with trisomy 18 in this heterogeneous population is irrelevant.

In the last paragraph of the letter, the authors claim that 30% of our cases with trisomy 18 had no other abnormalities on ultrasonography. This shows a misreading of our manuscript because there were 6 of 26, or 23%, with no other abnormalities.

In conclusion, a large prospective study would be most helpful to determine the risk of a fetus to have trisomy 18 when choroid plexus cysts are present without any other ultrasonographic abnormalities. In the absence of such a study, statistical analysis can be used to estimate this risk, as we have shown in our article. We have used the most accurate incidences for choroid plexus cysts in the general population available in the literature and believe that to date there is no reason to revise these numbers.

Beryl Benacerraf, MD
Diagnostic Ultrasound Associates, 333 Longwood Ave., Boston, MA 02115

Corrections

In the article by Simpson et al., "Does unexplained second-trimester (15 to 20 weeks' gestation) maternal serum α-fetoprotein elevation presage adverse perinatal outcome?" (Am J Obstet Gynecol 1991;164:829-36), Dr. Brian M. Mercer was erroneously deleted as a coauthor. The correct list of authors is: Joe Leigh Simpson, MD, Sherman Elias, MD, Cynthia D. Morgan, RN, MS, Richard N. Andersen, PhD, Lee P. Shulman, MD, Baha M. Sibai, MD, Brian M. Mercer, MD, and Amanda Skoll, MD.

In the article by Clark et al., "Position change and central hemodynamic profile during normal third-trimester pregnancy and post partum" (Am J Obstet Gynecol 1991;164:883-7), Fig. 1 is incorrect. The correct figure and legend are shown below.

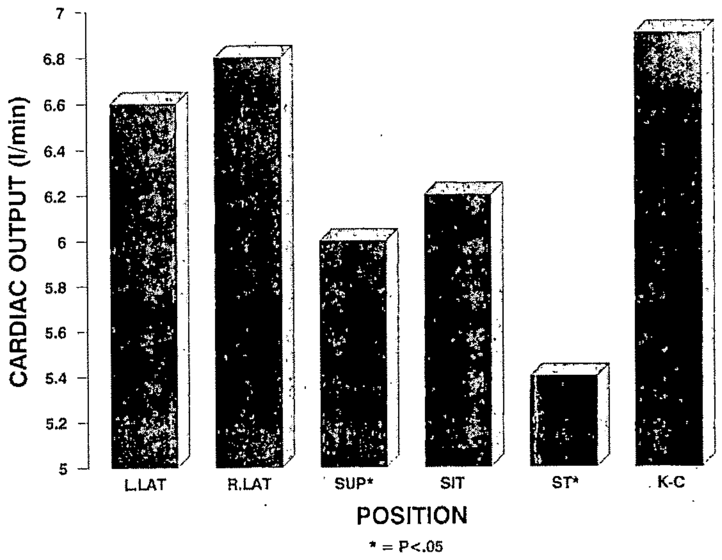

Fig. 1. Effect of position change on cardiac output during pregnancy. *L.LAT,* Left lateral; *R.LAT,* right lateral; *SUP,* supine; *SIT,* sitting; *ST,* standing; *K-C,* knee-chest.

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National Perinatal Association 1991 Annual Clinical Conference and Exposition, "Interdependence: The Perinatal Health Care Trend of the 90's," November 7-10, 1991, Boston, Massachusetts. For complete conference brochure, abstract application, or exhibitor information call NPA at (813) 971-1008 or Contemporary Forums at (415) 820-2800.

Pediatric and Adolescent Gynecology Postgraduate Course, October 10-11, 1991. Cosponsored by the Children's Hospital of Boston and Harvard Medical School, Department of Continuing Education, and held at The Inn at Children's and the Enders Auditorium at Children's Hospital in Boston, Massachusetts. Approximately 16 hours credit, Category I. For information contact: S. Jean Emans, MD, Division of Adolescent/Young Adult Medicine, Children's Hospital, 300 Longwood Ave., Boston, MA 02115.

Surgical Gynecology, October 18-19, 1991, The Ambassador West Hotel, Chicago, Illinois. For further information contact: Office of Continuing Medical Education, Northwestern University Medical School, 301 East Chicago Ave., Ward 1-004, Chicago, IL 60611-3008. Tel.: (312) 503-8533.

Breastfeeding in the 90's: Issues, Controversies and Solutions, Dr. Ruth Lawrence, keynote speaker, September 12, 1991, St. Louis, Missouri. For information contact: Mary Doerr, RN, St. Mary's Health Center, 6420 Clayton Road, St. Louis, MO 63117. Tel.: (314) 768-8119.

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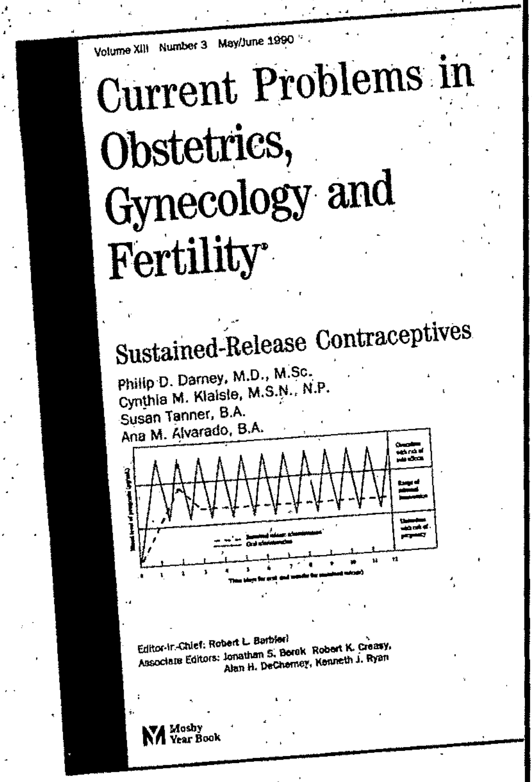

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DOOR COUNTY, WISCONSIN

BC/BE Obstetrician/Gynecologist to join established BC OB/GYN in hospital - based practice. Complete practice facilities available. New LDRP Birthing Center in modern, progressive 89 - bed community hospital. Competitive guaranteed salary and incentive package. Malpractice insurance. Attractive benefits.

Door County offers exceptional four season recreation along Lake Michigan shores, proximity to Milwaukee and Chicago, top rated schools and quality community life for family.

Send CV to: **Richard C. Murray, M.D.**
330 South 16th Place
Sturgeon Bay, WI 54235

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Send your curriculum vitae to **Irwin P. Goldstein, M.D., Associate Medical Director, SCPMG, Dept. 006, Walnut Center, Pasadena, CA 91188-8013.**

Or call: **1-800-541-7946**

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Obstetrician/ Gynecologists

Marshfield Clinic, a 350 physician multispecialty group practice is seeking BE/BC Obstsetrician/Gynecologists to join expanding regional referral centers in Chippewa Falls and Woodruff/Minocqua, Wisconsin. These are beautiful four-season recreational locations combining professional excellence with a family-oriented life style. Both have newly constructed Clinics with comparably modern hospitals. These practices provide an opportunity to enjoy the autonomy of a private practice with the financial and professional resources of a major multispecialty group. **Chippewa Falls** is located 1 1/2 hours from downtown Minneapolis/St. Paul and borders Eau Claire, Wisconsin, a city of nearly 80,000 which includes a major 4 year campus of the University of Wisconsin. Known as the "Lakeland Area," **Woodruff/Minocqua** is truly one of the most attractive vacation areas in the upper Midwest. An emphasis on quality practice and life style is combined with a very competitive salary and one of the finest fringe benefit packages in the country. For additional information, please send C.V. and references to: **David L. Draves, Director Regional Development, Marshfield Clinic**, 1000 North Oak Avenue, Marshfield, WI 54449, or call 1-800-826-2345, extension 5376.

MARSHFIELD CLINIC

OB/GYN WISCONSIN

Multi-specialty group in suburban Milwaukee seeks BC/BE Obstetrician/Gynecologist to join 8 physician Ob/Gyn department. Single site, single hospital practice. Competitive salary and fringe benefits.

Forward Inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI
53052-0427

The **Humana-Michael Reese HMO**, a large well-established HMO serving its members in 21 Health Centers throughout the Chicago Metro area, is seeking additional Board Certified/Eligible physicians in

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Physicians with the Humana-Michael Reese HMO enjoy a full practice with modern facilities and equipment as well as opportunities for teaching. All specialty facilities available.

A highly-competitive salary and excellent benefits complement a rewarding practice and lifestyle.

Interested applicants should address inquiries to:

Alfred Kendrick, M.D.
Medical Affairs
HUMANA-MICHAEL REESE HMO
2545 King Drive, Chicago 60616
or call (312) 808-4551

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OB/GYN

A 180-bed County Hospital located in the San Francisco Bay Area and serving a Medi-Cal and HMO population is seeking a BE/BC OB/GYN. Duties will include active GYN surgery service and outpatient GYN and Prenatal Clinics; supervision of U.C. Davis-affiliated residents in a 150 delivery/month OB service. Night call will be shared with four others and can be taken from home. Competitive salary and benefits. Send C.V. to: **D. Kent Hobert, M.D., Department OB/GYN, Merrithew Memorial Hospital, 2500 Alhambra Avenue, Martinez, CA 94553.**

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Excellent salary and benefits package for qualified candidates. Physicians will be granted academic rank based upon individual credentials. Participation available in successful physician private practice plan. Send c.v. or call: **Jasmin Moshirpur, MD, Director of Obstetrics & Gynecology/Associate Dean for Elmhurst Affairs, Room #C8-13, (718) 830-1340/1348.**

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CHIEF, OBSTETRICS/GYNECOLOGY

Newton-Wellesley Hospital, a 340-bed Tufts affiliated community teaching hospital, located less than 10 miles west of Boston, is conducting a search for a Chief to head the Department of Obstetrics & Gynecology. This position is full-time including practice opportunities.

Candidates must be board certified in Obstetrics and Gynecology and have strong clinical interests and skills, a commitment to family-centered patient care, management skills, and an ability to lead an active educational program geared to the needs of a rapidly growing department. Interest and expertise in one of the subspecialties of Obstetrics and Gynecology is preferred.

There are currently 3600 deliveries per year at the Newton-Wellesley Hospital, with continued growth anticipated. The Maternity Facility consists of a 20-bed LDRP Unit, 6 LDR suites, 2 C-section/high risk delivery suites, 2-bed PACU and 24-bed post partum unit, and an antenatal testing unit. This service is supported by a 12-bed, Level II, special care nursery, as well as full-time neonatology and anesthesia coverage.

The successful candidate will be responsible for the ongoing clinical, administrative, and teaching activities for the department, as well as for the direction of its active growth and development.

Interested candidates should forward their curriculum vitae to: Joel Rubenstein, M.D., Chairman, Search Committee, Newton-Wellesley Hospital, 2000 Washington Street, Newton, MA 02162.

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Newton-Wellesley Hospital

Oakwood Hospital

Department of Obstetrics and Gynecology Resident Education Program Director

We seek a Program Director for our fully accredited, independent, 16 person, Resident Education Program in Obstetrics and Gynecology. Nine full-time faculty members. Three well established divisions directed by full-time, certified, subspecialists. Annually there are 4,500 deliveries and 3,500 gynecologic surgical procedures at our 615-bed, acute tertiary care hospital.

Interested candidates should send their curriculum vitae to: **Martin Farber, M.D., Senior Vice President, OAKWOOD HOSPITAL, 18101 Oakwood Boulevard, P.O. Box 2500, Dearborn, MI 48123-2500, or phone (313) 593-7803** for further information. Equal Opportunity Employer.

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Private Practice

Obstetrician Gynecologist

Private practice opportunity for BC/BE Obstetrician/Gynecologist to join or affiliate with a six member Board Certified OB/GYN group in Sioux Falls, South Dakota.

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Sioux Falls is an attractive city with a population of 100,000 plus. Our community has a strong economic base, growing population and a progressive educational system including two local colleges. Medical School appointment possible.

Excellent salary, fringe benefits and incentives.

Please send curriculum vitae in confidence to:
Carl Hinson
Obstetrics & Gynecology, Ltd.
1200 South Euclid Avenue Suite 204
Sioux Falls, South Dakota 57105
Please call:
1-800-468-3333 for informational packet.

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FACULTY POSITION

Reproductive Endocrinology and Infertility

The Department of Obstetrics and Gynecology at the Ohio State University College of Medicine is seeking a board-certified or board-eligible physician in Reproductive Endocrinology and Infertility for a faculty position at the rank of assistant professor. An excellent opportunity exists for research, teaching and clinical practice.

Send curriculum vitae and/or contact Steven G. Gabbe, M.D., Professor and Chairman, or Moon H. Kim, M.D., Professor and Division Director, Department of Obstetrics and Gynecology, Ohio State University Hospitals, 535 Means Hall, 1654 Upham Drive, Columbus, Ohio 43210. Telephone (614-293-8511).

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NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

Kaiser Permanente offers competitive salaries and excellent benefits including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE

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Faculty Position
Maternal Fetal Medicine
Southern Illinois University School of Medicine
Springfield, Illinois

The Department of Obstetrics and Gynecology at Southern Illinois University School of Medicine, Springfield, Illinois is expanding the Division of Maternal Fetal Medicine and is seeking one new faculty at the Assistant or Associate Professor level. The division serves as a state-of-the-art perinatal referral center for central and downstate Illinois and is responsible for providing tertiary care to high risk obstetrical patients, including antenatal testing, perinatal ultrasonography (including Doppler), and fetal evaluation and therapy with planned expansion of its prenatal diagnostic services to include CVS. Division faculty are also responsible for the development of educational curriculum and delivery of instruction to medical students, residents, and fellows. The division is committed to research activities pertinent to maternal fetal medicine, and research opportunities are available. Certification or eligibility in the subspecialty is required.

Send Curriculum Vitae to: Robert D. Hilgers, M.D., Professor & Chairman, Department of OB/GYN, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794. Phone: (217) 782-8247. Or send inquiries to Robert C. Kaufmann, M.D., Chief, Division of Maternal Fetal Medicine.
Phone: (217) 785-5540

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for four (4) full-time faculty positions for a new women's health center and academic program at its Milwaukee Clinical Campus.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5500 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin and St. Luke's Medical Center in Milwaukee.

Qualifications include board candidacy or certification in obstetrics and gynecology and a commitment to teaching residents, medical students, and clinical research. Qualifications for the four positions for GENERAL OB/GYN can take into consideration special individual interests. Special expertise of interest for the department are:

- STATEWIDE FAMILY PLANNING AND AMBULATORY CARE (Part-time medical director, Planned Parenthood)
- OPERATIVE LAPAROSCOPY, HYSTEROSCOPY
- UROGYNECOLOGY
- RESIDENCY/MEDICAL STUDENT EDUCATION
- OB/GYN ULTRASOUND

Private practice opportunities are also available in an associated offsite multispecialty clinic and all faculty members participate in a group practice at the institution. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue, H4/564 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

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FACULTY POSITION
DIVISION DIRECTOR
MATERNAL-FETAL MEDICINE

- ANTENATAL UNIT
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- PREMIER NEWBORN INTENSIVE CARE UNIT
- 7 NEONATOLOGISTS
- 24 HR. IN HOUSE OBSTETRICAL ANESTHESIA
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- TEACHING RESIDENTS & STUDENTS

The Department of Obstetrics & Gynecology at the University of Kansas School of Medicine-Wichita is accepting applications for the position of Director of the Division of Maternal-Fetal Medicine. This represents a unique opportunity to have the best features of both academic medicine and private practice.

Please send curriculum vitae to:

Daniel K. Roberts, M.D., Ph.D.
Professor & Chairman, Dept. of Ob/Gyn
University of Kansas School of Medicine-Wichita
HCA-Wesley Medical Center
550 N. Hillside
Wichita, KS 67214

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STAFF PERINATOLOGIST

Our **Maternal Fetal Medicine Section** is seeking to increase its current staff of six perinatologists and one geneticist, to cover our rapidly expanding responsibilities in the fields of prenatal diagnosis and high risk obstetrics.

PENNSYLVANIA HOSPITAL, the Nation's First Hospital, is a tertiary care center, located in the historic Society Hill Section of Philadelphia.

Our section provides perinatal consultation, ultrasound evaluation, and fetal testing to an extensive referral network of surrounding institutions. Pennsylvania Hospital is also the site of the International PUBS Registry, has an active prenatal diagnosis program involving CVS and amniocentesis, and a committment to teaching with four Maternal Fetal Medicine Fellows.

Faculty appointment at the **University of Pennsylvania School of Medicine** is available. Malpractice insurance coverage and a liberal benefit package are offered. All applicants must be board certified or board eligible.

For more information, please write or contact:

Stuart Weiner, M.D.
Section Chief, Maternal Fetal Medicine
Department of Obstetrics/Gynecology
Pennsylvania Hospital
800 Spruce Street
Philadelphia, PA 19107
(215) 829-5808

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DIRECTOR OF MATERNAL-FETAL MEDICINE

Albert Einstein Medical Center (AEMC), a 600+ bed tertiary care teaching hospital, is seeking a board certified maternal-fetal medicine subspecialist as Director of the Division of Maternal-Fetal Medicine to develop and direct the comprehensive perinatal program in this institution. AEMC is a major referral center in north Philadelphia supporting numerous post-graduate medical education programs, including a fully accredited residency program in obstetrics and gynecology. The Department of Obstetrics and Gynecology has a long-standing tradition of excellence in healthcare delivery and supports a variety of clinical research and educational activities. The Division of Maternal-Fetal Medicine serves as a tertiary referral center within the metropolitan Philadelphia area. Its service includes maternal intensive care unit, maternal transport program, high risk obstetrics, genetic counseling, diagnostic genetic procedures (amniocentesis, CVS, cordocentesis), targeted (level II) obstetrical ultrasound and comprehensive antenatal fetal surveillance. Our Department is supported by a Level III NICU and a Cytogenetic Laboratory. There is a strong institutional support and emphasis in research with funds and laboratory space available for basic research. A construction project is under way for a new labor and delivery suite and a comprehensive fetal diagnostic center. We are also planning to develop a maternal-fetal medicine fellowship training program with Temple University.

AEMC is a major affiliate with Temple University. Academic appointment is available for all full-time AEMC faculty. Highly competitive salary and very attractive fringe benefits are included. If you are interested in this position or would like additional information about this unique opportunity, please write to Sze-ya Yeh, M.D., Chairman, Department of Obstetrics and Gynecology, Albert Einstein Medical Center, 5401 Old York Road, Klein #410, Philadelphia, PA 19141. Telephone (215) 456-6993, FAX (215) 456-6271.

PERINATAL ASSOCIATES OF NORTHERN CALIFORNIA

Are you a clinical perinatologist? There is still time to join four perinatologists and one geneticist-OB/GYN looking for one more perinatologist who: desires superior environment for patient care, in a private, modern, level 3 facility doing 6,000 deliveries a year with superior perinatal outcome; likes personal, direct hands-on patient care; needs PUBS, IUTxF, CVS and complicated pregnancies, level 2 ultrasound; demands partners, not superiors; wants to live in clean, warm, not humid, snow-free (but 60 minutes from skiing) Sacramento, California; feels one's family has priorities, and is committed to the private practice of medicine. For more information, call (916) 733-1755, extension 2773.

Hanns C. Haesslein, M.D.
Perinatal Associates
of Northern California
5301 F Street
Sacramento, California 95819

Gynecologic Oncologist Faculty Position

The James H. Quillen College of Medicine of East Tennessee State University (ETSU) is seeking a board certified/eligible Gynecologic Oncologist. This position offers an excellent opportunity combining research, teaching and private practice with a faculty position at the Assistant/Associate Professor level. Salaries are very competitive and fringe benefits are excellent. The Medical School is located in the Tri-Cities area of Northeast Tennessee in a beautiful section of the Appalachians and within seeing distance of the Blue Ridge Mountains. The area has excellent hunting, fishing, and camping with multiple ski resorts at your doorstep. The Tri-Cities combine good schools and rural living in a safe, beautiful environment. Yet, the medical referral base consists of 1.3 million people within a 50 mile radius of the Medical School. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman, Dept. OB/GYN
James H. Quillen College of Medicine
East Tennessee State University
P.O. Box 19570A
Johnson City, TN 37614-0002

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Finger Lakes Region
Ithaca, New York

Unique opportunity for patient oriented physician in comprehensive OB-GYN service, building on established patient base of 6,000 patients. To include out-patient gynecology, obstetrics, surgery, infertility, and abortions. First call protection from nurse-midwives. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Summer/Fall 1991.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

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D. Henry Ruth, M.D.
Ruth, Green & Associates
213-597-0610
One World Trade Center
Box 32133
Long Beach, CA 90832
217 Taylor Avenue, Beaver, PA 15009

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
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Maternal Fetal Medicine Fellowship
Department of Obstetrics and Gynecology

Southern Illinois University School of Medicine
Springfield, Illinois

The Department of Obstetrics and Gynecology at Southern Illinois University School of Medicine, Springfield, Illinois has one first year Fellowship position available in Maternal Fetal Medicine beginning July 1, 1992. This is a two year accredited Fellowship program in which both clinical and research training are emphasized. Clinical material is drawn from the 3000 deliveries/year at St. John's Hospital Perinatal Center where fellows participate in a supervised, in-depth experience in management and treatment of medical and obstetrical complications with emphasis on antenatal diagnostic procedures and ultrasound techniques. This flexible program incorporates a minimum six month period of full time research and accommodates various clinical/research objectives based on the fellow's individual goals. Fellows are encouraged to present papers and to represent the Maternal Fetal Medicine Division at national scientific meetings.

Send Curriculum Vitae to: Robert C. Kaufmann, M.D., Chief, Division of Maternal Fetal Medicine, Department of OB/GYN, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, IL 62794.
Phone: (217) 782-8240

SIU

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PORTLAND, OREGON

Outstanding opportunity for BC/BE OB/GYN to join rapidly expanding single specialty group of four physicians in Portland's most desirable suburb. Attractive and growing population base for both OB and GYN services has created immediate demand for **two** physicians. Hospital services include 12 LDR's, level II nursery and 3,000 deliveries per year. Level III nursery available in the community. Outstanding array of surgical equipment and support.

Send CV and references to:

Joanne Rudoff, M.D.
4035 SW Mercantile Drive
Lake Oswego, Oregon 97035
(503) 292-0171

MORRIS COUNTY, NJ
Less than an hour from NYC

Two opportunities available. A 16-month old progressive, growing multi-specialty group desires OB/GYN. Group has 4 FP and all specialties except OB/GYN.

An established 3-person OB/GYN practice is looking to expand. Doctors have two offices and a strong community reputation.

Area boasts large child bearing population and provides an excellent environment for private practice. Positions offer attractive salary and benefit packages.

Nearby St Clares • Riverside Medical Center (400+ beds) recently opened The BirthPlace with 18 private postpartum rooms, 2 dedicated delivery/c-section rooms and 6 LDR suites. 1992 graduates are encouraged to respond.

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ST CLARES RIVERSIDE
MEDICAL CENTER

Send CV to:
Steve Alderson
22 Bloomfield Avenue
Denville, NJ 07834
(201) 625-4602

Maternal Fetal Medicine Subspecialist
Faculty Positions

The James H. Quillen College of Medicine of East Tennessee State University (ETSU) is seeking board certified/eligible Maternal-Fetal Medicine (Perinatology) subpecialists. These positions offer an excellent opportunity combining research, teaching and private practice with a faculty position at the Assistant/Associate Professor level. Salaries are very competitive and fringe benefits are excellent. The Medical School is located in the Tri-Cities area of Northeast Tennessee in a beautiful section of the Appalachians and within seeing distance of the Blue Ridge Mountains. The area has excellent hunting, fishing, and camping with multiple ski resorts at your doorstep. The Tri-Cities combine good schools and rural living in a safe, beautiful environment. Yet, the medical referral base consists of 1.3 million people within a 50 mile radius of the Medical School. Send curriculum vitae to:

Frederick R. Jelovsek, M.D.
Professor and Chairman, Dept. OB/GYN
James H. Quillen College of Medicine
East Tennessee State University
P.O. Box 19570A
Johnson City, TN 37614-0002

ETSU is an AA/EOE

State University of New York
Health Science Center
Syracuse

MATERNAL-FETAL MEDICINE SUBSPECIALIST
ASSISTANT PROFESSOR/ ASSOCIATE PROFESSOR

The Department of Obstetrics and Gynecology of the SUNY Health Science Center at Syracuse is actively recruiting a full-time Perinatologist for its Division of Maternal-Fetal Medicine.

The teaching hospital serves as the only Tertiary Care Center for the Regional Perinatal Program encompassing the 15 counties of Central New York with a population of 2.3 million.

Excellent opportunity to participate in teaching in a University based setting and collaborate in ongoing research in clinical Perinatology, Ultrasound, and Prenatal Diagnosis.

TOP SALARY, ATTRACTIVE BENEFITS PACKAGE, AND PROTECTED RESEARCH TIME.

Board eligibility certification in Maternal-Fetal Medicine is required.

Interested candidates should send curriculum vitae to:

Robert K. Silverman, M.D.
Division of Maternal-Fetal Medicine
SUNY Health Science Center at Syracuse
Perinatal Center
725 Irving Avenue, Suite 115
Syracuse, NY 13210
(315) 470-7196

SUNY Health Science Center is an equal opportunity, affirmative action employer.

FACULTY POSITIONS
GENERAL OBSTETRICS AND GYNECOLOGY

The University of North Carolina at Chapel Hill, Department of Obstetrics and Gynecology, in conjunction with the Wake Medical Center, Raleigh, North Carolina, and the Wake Area Health Education Center is seeking two board eligible or active candidates in General Obstetrics and Gynecology. One position will require special interest in gynecological surgery and the other special interest in obstetrics.

Wake Medical Center is located in Raleigh, the State Capitol, approximately 40 minutes from Chapel Hill, and is a 560-bed comprehensive tertiary care center with over 4000 deliveries and 1500 surgical procedures each year.

The positions are in the Wake AHEC OB/GYN Department which has four other full-time obstetricians and gynecologists.

Excellent opportunity for clinical research and teaching of the UNC/Chapel Hill Ob/Gyn residents and medical students rotating through Wake Medical Center at all times. Collaboration with the Department of Obstetrics and Gynecology at Chapel Hill is encouraged and anticipated.

Salary and academic rank commensurate with training and experience. Superb fringe benefits package.

Write or contact:

Juan L. Granados, M.D.
Wake AHEC OB/GYN Teaching Director
Wake Medical Center
P.O. Box 14465
Raleigh, NC 27620-4465
(919) 250-8535

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FACULTY OPENINGS IN OBSTETRICS & GYNECOLOGY FOR GENERALISTS/PERINATOLOGISTS

Full time faculty positions at the University of South Alabama, Department of Obstetrics & Gynecology. Excellent opportunity for teaching, research and clinical service. Individuals must be board eligible or certified in Specialty/Sub-specialty. The academic rank and salary will be dependent upon the training and experience of the applicant. These positions are available immediately.

Please contact:

Ian H. Thorneycroft, Ph.D., M.D.
Professor and Chairman
Department of Ob-Gyn
University of South Alabama
College of Medicine
2451 Fillingim Street
Mobile, Alabama 36617
(205) 470-5815

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REPRODUCTIVE ENDOCRINOLOGIST

The University of Vermont College of Medicine, Department of Obstetrics and Gynecology, seeks a fourth Board Certified/Board Eligible Reproductive Endocrinologist to join an active, academic division. This tenure track appointment will allow ample time and opportunity for teacching and the pursuit of independent research interests. The division has a well established, accredited RE fellowship, proven research productivity, a successful IVF program and a busy endoscopic surgical service. Salary and rank will be commensurate with the successful candidate's experience. Applications will be accepted until position is filled. Please send curriculum vitae to:

Daniel H. Riddick, M.D., Ph.D.
Chairman, Department of Obstetrics and Gynecology
Medical Center Hospital of Vermont
111 Colchester Avenue
Burlington, VT 05401

The University of Vermont is an Equal Opportunity/Affirmative Action Employer. Applications from women and people from diverse racial, ethnic, and cultural backgrounds are encouraged.

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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.
CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert. ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

‡Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

§An increase in blood pressure has been reported in women taking oral contraceptives.

1. Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med.* 1989;34(8):540-542.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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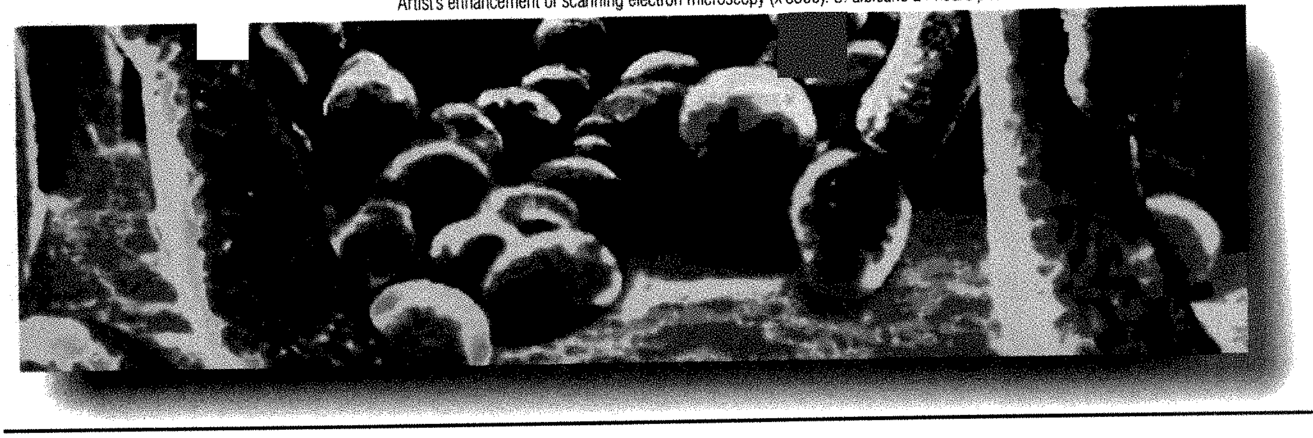
In *vulvovaginal candidiasis,* the proven efficacy of 3-day therapy* with FEMSTAT Prefill means a microbiological cure in 95% of patients.[1]†

1. Droegemueller W, Adamson DG, Brown D, et al: Three-day treatment with butoconazole nitrate for vulvovaginal candidiasis. *Obstet Gynecol* 1984;64:530-534. *In pregnant patients (second and third trimesters only), therapy should be extended to six days. †Microbiological cure in 95% of patients (n = 126) eight days following FEMSTAT 3-day therapy. ‡Micrographs on file, Syntex Laboratories, Inc. Please see next page for brief summary of prescribing information. © 1991 Syntex Laboratories, Inc. 813-12-189-91

Artist's enhancement of scanning electron microscopy (x 3500): *C. albicans* 24 hours posttreatment with butoconazole nitrate.‡



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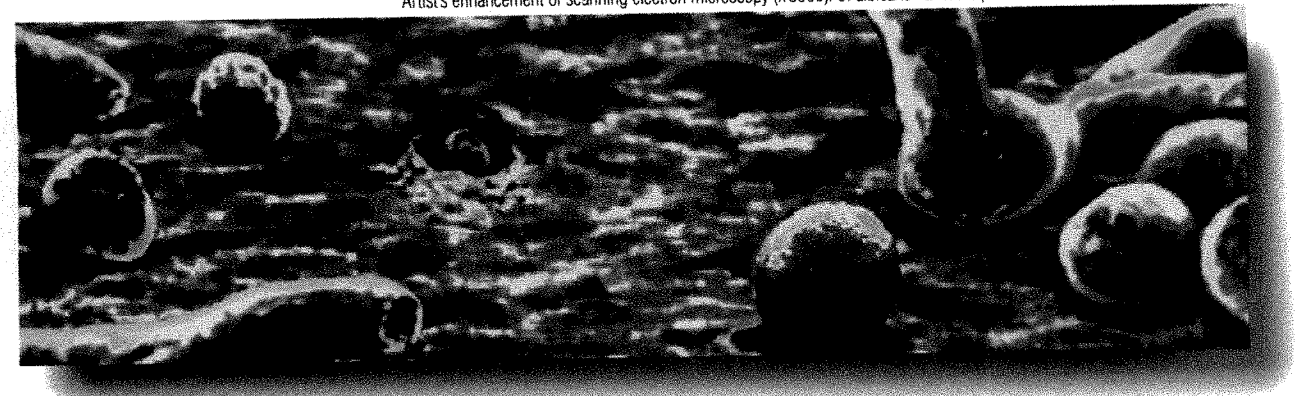

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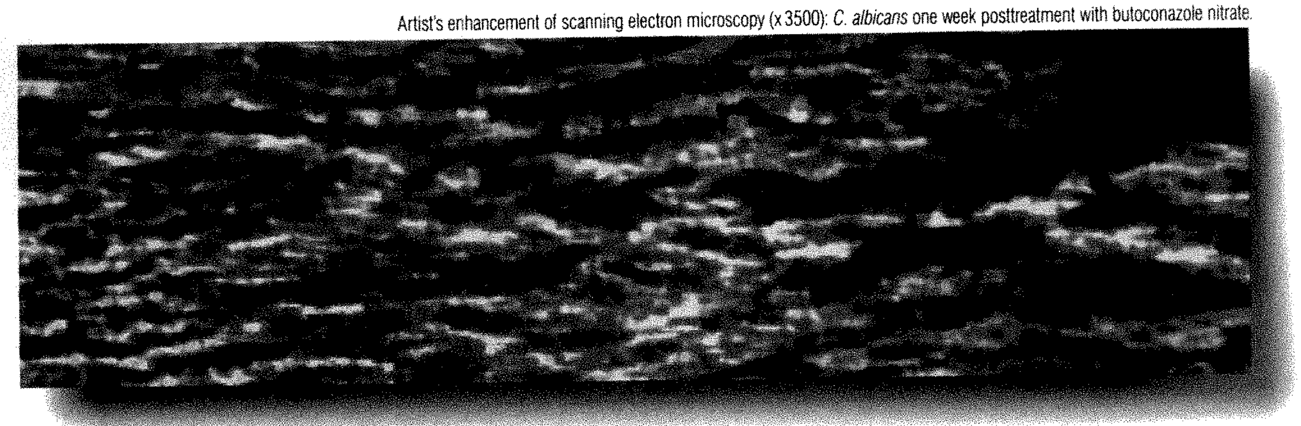

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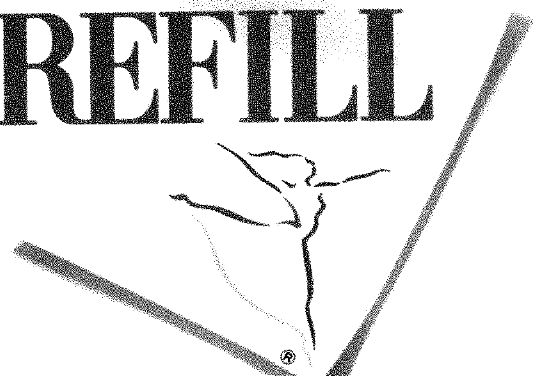

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INDICATIONS: FEMSTAT® (butoconazole nitrate) vaginal cream 2% is indicated for local treatment of vulvovaginal mycotic infections caused by Candida species. Confirm the diagnosis by KOH smears and/or cultures. FEMSTAT can be used with oral contraceptives and antibiotics. It is effective in non-pregnant women and during the second and third trimesters of pregnancy.

CONTRAINDICATIONS: FEMSTAT is contraindicated in patients hypersensitive to any of the ingredients.

PRECAUTIONS: General: If clinical symptoms persist, repeat microbiological tests to rule out other pathogens and confirm the diagnosis. Discontinue drug if sensitization or irritation occurs.

Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

Nursing Mothers: Use with caution.

Pediatric Use: Safety and efficacy have not been established.

ADVERSE REACTIONS: Vulvar/vaginal burning in 2.3% of patients, vulvar itching in 0.9%, discharge, soreness, swelling, itching of fingers each in 0.2%. Complaints caused 1.6% to discontinue drug.

DOSAGE AND ADMINISTRATION: Non-pregnant Patients: The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for three days. Treatment can be extended for an additional three days if necessary.

Pregnant Patients (second and third trimesters only): The recommended dose is one applicatorful of cream (approximately 5 grams) intravaginally at bedtime for six days.

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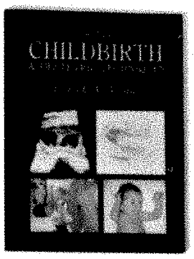

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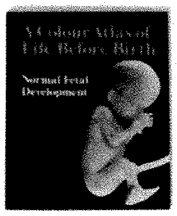

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*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.

See brief summary on adjacent page.

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Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5). 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes of the vagina and cervix. Although these changes are histologically benign, it is not known whether this condition is a precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract. Although similar data are not available with use of other estrogens, it cannot be presumed they would not induce similar changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been reported with use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in risk of limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pregnancy, or attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that risk of limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female sex hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is considerable evidence that estrogens are ineffective for these indications, and there is no evidence from well-controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other types of polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Embryos with these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontaneous abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any patient who has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first missed period, and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, the patient should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy should be discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use an alternate form of contraception for a period of time before attempting to conceive. Many clinicians recommend 3 months, although no precise information is available on which to base this. The administration of progestogen-estrogen combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users of OC's and estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In one of the other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a significant percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully observed while on OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three studies were performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight increase in triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some women, hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women with hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence in users increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalence in the 1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previously have had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hypertension that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is recurrent, persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patients discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional causes should be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagnostic measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change to another OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in minimizing menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic disease. Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycles may have a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these preexisting problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation, possibly prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity and quality of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of mothers on OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has been weaned.

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of OC's. Pretreatment and periodic physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be prescribed for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-progestogen preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients becoming significantly depressed while on OC's should stop OC's and use an alternate method to try to determine whether the symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, such as convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past history of jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's should be discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and should be administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may result in a relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depressed by OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate deficiency increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping OC's, she may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9. The pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine- and liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobromophthalein retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating total-thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG: free T4 concentration is unaltered. d. Decreased pregnanediol excretion. e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been associated with concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, phenytoin sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use of OC's (see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral hemorrhage, hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an association between the following conditions and use of OC's although additional confirmatory studies are needed: mesenteric thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related. Nausea and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of patients during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally. Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, change in menstrual flow; dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinuance of treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and secretion; change in weight (increase or decrease); change in cervical erosion and cervical secretion; possible diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leiomyomata; rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening), intolerance to contact lenses. The following adverse reactions have been reported in users of OC's, and the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in libido, chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndrome.

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of OC's by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly as directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cycle of medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutive days of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)

Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contraceptive method until she has taken a tablet daily for 7 consecutive days. For full details on dosage and administration see prescribing information in package insert.

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August
1991

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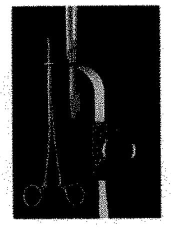

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(cefotetan disodium)
In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD), pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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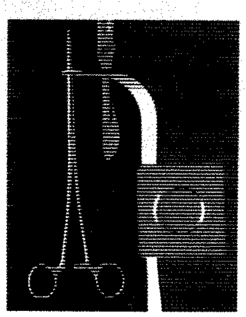

Count on

CEFOTAN® IM/IV

(cefotetan disodium)

In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
(FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.)

INDICATIONS AND USAGE

Treatment: CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis*, and *Proteus* sp (which may include the organisms now called *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), *E coli*, *Proteus mirabilis*, and *Serratia marcescens*.*

Skin and Skin Structure Infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus* species (excluding enterococci), *E coli*, *Klebsiella pneumoniae*, and *Peptococcus** and *Peptostreptococcus* sp.*

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*, *Streptococcus* species (excluding enterococci), group B streptococci, *E coli*, *Proteus mirabilis*, *Neisseria gonorrhoeae*, *Bacteroides* species (excluding *B distasonis*, *B ovatus*, and *B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species).

Intra-abdominal Infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B distasonis*, *B ovatus*, and *B thetaiotaomicron*), and *Clostridium* species.*

Bone and Joint Infections caused by *Staphylococcus aureus*.*

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

Note: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Prophylaxis: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes should also be considered.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

During postmarketing experience with CEFOTAN, agranulocytosis, anaphylactic reactions, fever, hemolytic anemia, leukopenia, prolonged prothrombin time with or without bleeding, pseudomembranous colitis, and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

Treatment: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

Prophylaxis: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

Impaired Renal Function: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:}\quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:}\quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

Manufactured for
STUART PHARMACEUTICALS
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Wilmington, Delaware 19897 USA

Rev. S 05/91

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Edmond A. Dowling, MD, Frederick Cone, BS, George W. Creasy, MD, and
Michael E. Kafrissen, MD, MPH
Pittsburgh, Pennsylvania, Mobile, Alabama, and Raritan, New Jersey

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Christopher P. Crum, MD
Charlottesville, Virginia

Invasive squamous cell carcinoma of the vulva, unlike vulvar intraepithelial neoplasia, is only infrequently associated with human papillomavirus deoxyribonucleic acid.

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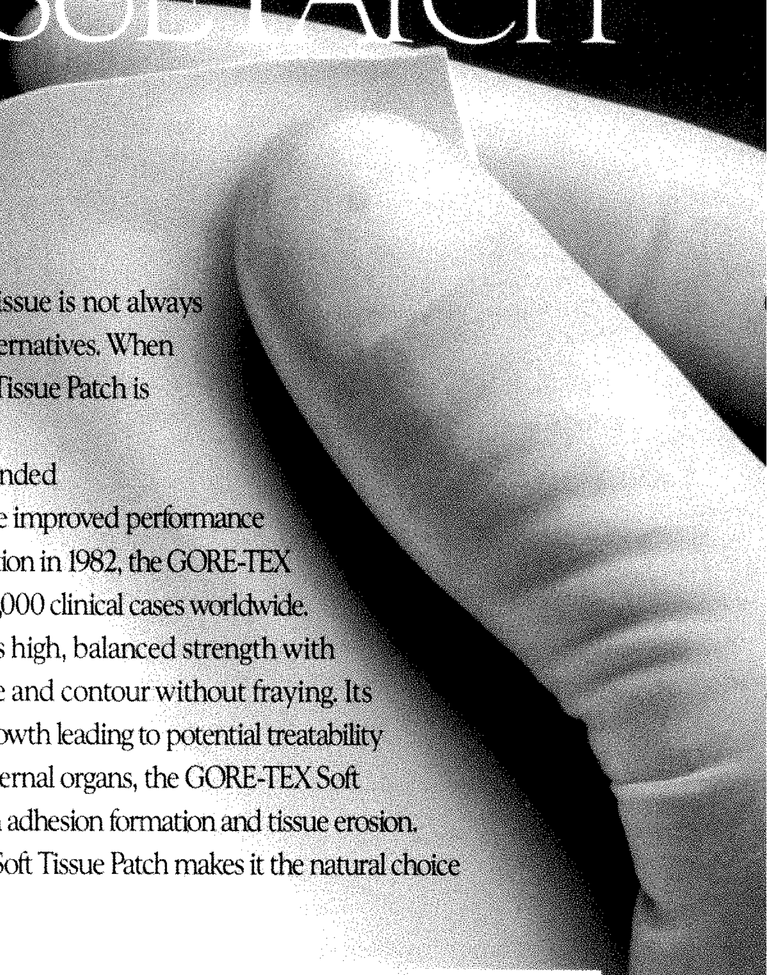

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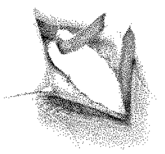
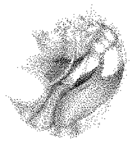
- Related Pediatric Reconstructions





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Rita O. Pucci, MD, and Randolph W. Seed, MD, PhD
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Clark Brittain, Maj, MC, USA, Jay W. Carlson, Cpt, MC, USA, Dan L. Gehlbach, Maj, MC, USA, and Andrew W. Robertson, Maj(P), MC, USA
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Stacie Bosnyak, MD, Joseph M. Baron, MD, and James Schreiber, MD
Chicago, Illinois

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Douglas S. Richards, MD, Robert M. Abrams, PhD, Kenneth J. Gerhardt, PhD, and Mary E. McCann, MA
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Robert D. Tucker, PhD, MD, Jo Ann Benda, MD, Ali Mardan, PhD, MD, and Tibor Engel, MD
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Hong-Nerng Ho, MD, Thomas J. Gill III, MD, Chang-Yao Hsieh, MD, Yu-Shih Yang, MD, and Tzu-Yao Lee, MD
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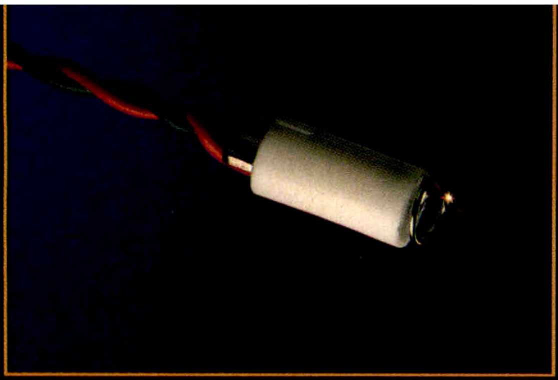

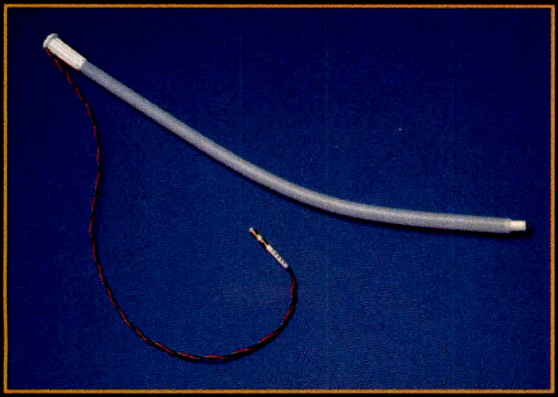

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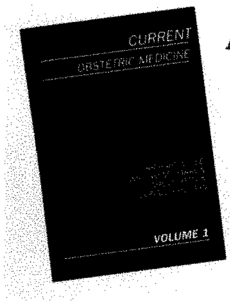

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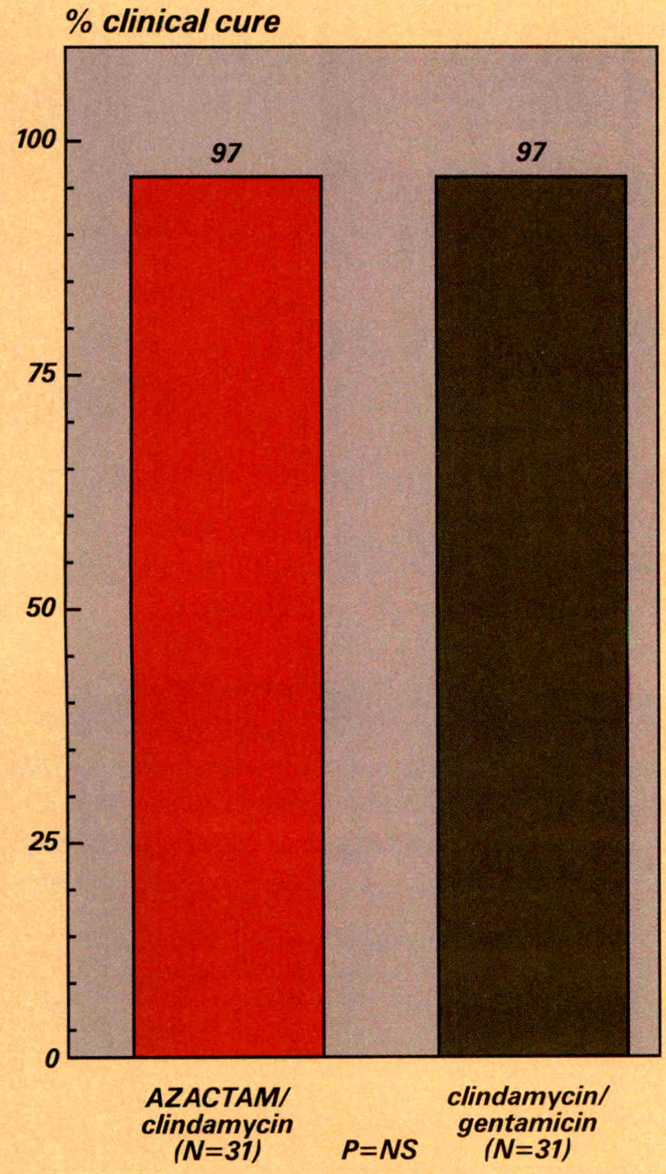

A randomized, comparative trial of AZACTAM versus gentamicin, each with clindamycin, for the treatment of endometritis after delivery.[1]

No universal criteria exist that may satisfy a single definition of "cure." Use of this term may vary between clinical settings and investigators, and the reader is urged to consult the reference listed.

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delivers...

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*Due to susceptible organisms.

†Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

‡Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. See full prescribing information for recommendations on patients with immediate hypersensitivity reactions to penicillins.

Following IV administration, the most commonly seen adverse reactions occurred in less than 2% of patients, including local reactions, diarrhea, nausea/vomiting, and rash.

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- *May be admixed in the same bag with clindamycin.*
- *May be used in penicillin-allergic patients.*[‡]
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There are no adequate and well-controlled studies in pregnant women. AZACTAM should be used in pregnant women only if clearly needed.

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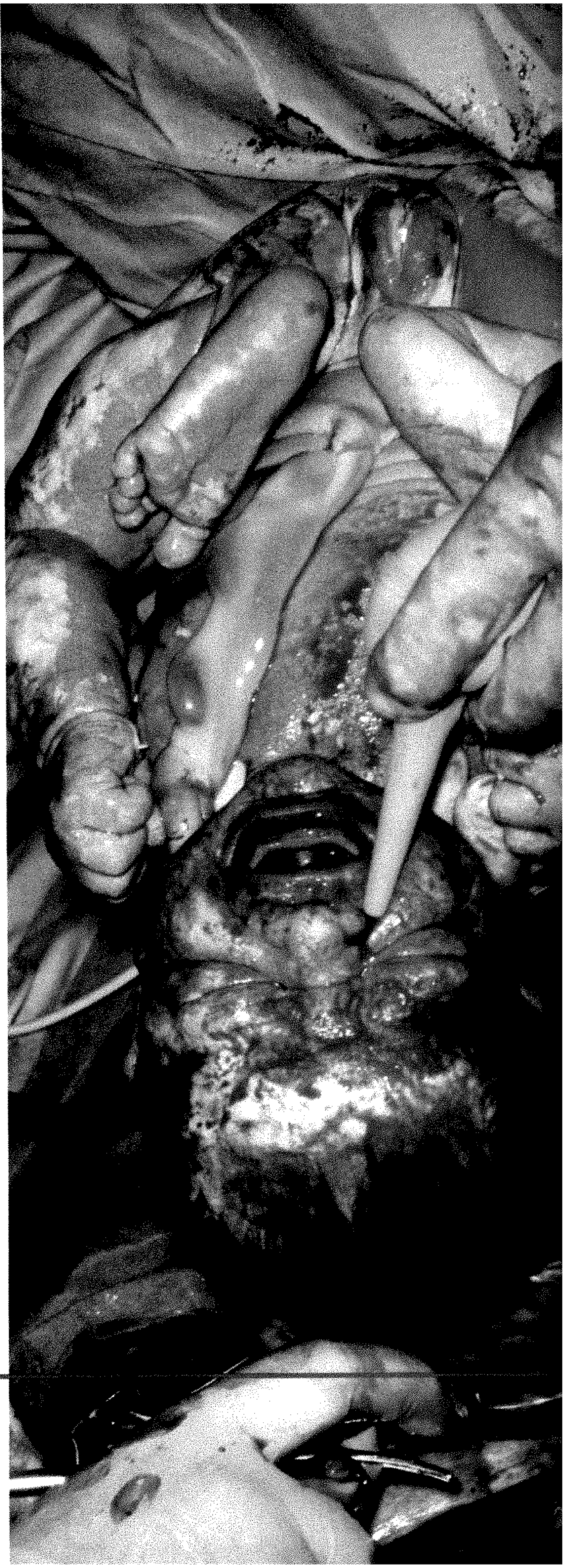

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AZACTAM® FOR INJECTION
Aztreonam For Injection

DESCRIPTION–AZACTAM (aztreonam, Squibb) is the first member of a new class of antibiotics classified as monobactams. AZACTAM is a totally synthetic bactericidal antibiotic with activity against a wide spectrum of gram-negative aerobic pathogens. The monobactams, having a unique monocyclic beta-lactam nucleus, are structurally different from other beta-lactam antibiotics.

AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca**, *Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis**, *Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae**, *Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae**, *Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organisms(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

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*Efficacy for this organism in this organ system was studied in fewer than ten infections.

References: *1. Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* ***2.*** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* ***3.*** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

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American Journal of Obstetrics and Gynecology
Founded in 1920
volume 165 number 2 AUGUST 1991

TRANSACTIONS OF THE FIFTY-THIRD ANNUAL MEETING OF THE SOUTH ATLANTIC ASSOCIATION OF OBSTETRICIANS AND GYNECOLOGISTS

Our shield: Competence with compassion

Presidential address

M. Tucker Laffitte, Jr., MD
Columbia, South Carolina

Our organization has been blessed with a wonderful heritage. The "town and gown" leadership has worked well. Today, as never before, we must act in concert with other professions and other public bodies in an effort to deliver excellent health care in an environment we all enjoy.

Improved patient care has always been the thrust of our education, but in recent years the cost of this care has become a dominant factor. The excellent quality of health care in this country is recognized throughout the world, but the public and business community have become alarmed by the ever-escalating cost.

Of equal concern is the stated lack of medical care available to a significant number of our population. Our aging society poses additional stress on the so-called "health-care dollar." In 1980, those over 65 years of age numbered 25 million. By the year 2025, that number will increase to 60 million. This fact alone reminds us of the importance of preventive medical health care. Ideally, we would all like to die in a state of good health.

The advice we give does have an impact in improving the health of women. Teenage counseling and education lower the incidence of sexually transmitted disease and unplanned pregnancies. The yearly Papanicolaou smear has almost eliminated death from cancer of the cervix. Screening for diabetes and other metabolic conditions has allowed for earlier diagnosis and treatment. The detrimental effects of tobacco and alcohol are being emphasized. Genetic counseling has allowed patients to anticipate obstetric outcome. Estrogen replacement in postmenopausal patients lowers mortality by decreasing osteoporosis and improves the quality of life. Improved diet and exercise lower the incidence of cardiovascular disease. Mammography gives us a tool that lowers the mortality from breast cancer. Fewer prescriptions from us decrease the incidence of drug dependence. Informing our patients will require some adjustment on our part as these endeavors are time-consuming.

In 1989 *U.S. News and World Report* rated United States hospitals as to which were best. I suspect that we too are being rated. Do we improve patient care? Do we influence costs? Do we influence quality? Do we in general foster and stimulate improvements in the health care of women who properly come within the parameter of obstetrics and gynecology? These are the purposes that were stated more than 50 years ago by the founders of this organization. These continue to be our purpose; however, many changes have occurred in our society and our profession since its organization.

Before World War II, women were rarely included in our work force or professional ranks. Two-parent families were traditional. Before 1973, elective abortion was illegal. Sexuality was a private matter. Homosexuality was an abnormal state, and sex within marriage was hallowed. There was respect for our institutions: the church, the legal profession, and the medical profession. All have suffered a great deal in the last few decades.

It is said that one third of our population is deprived of adequate medical care. What is our role in assuring care to all members of our society? The public has

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Presented at the Fifty-third Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 27-30, 1991.

Reprint requests: M. Tucker Laffitte, Jr., MD, Columbia OB-GYN, 1333 Taylor St., Suite 5-F, Columbia, SC 29201.

6/6/30070

stepped in to "fill this so-called void." Politicians have become very vocal and great amounts of money have been spent. To maintain our credibility, we should be advocates for keeping our patients' costs down and avoiding business partnerships that are profit-driven.

Technology has allowed many women to conceive and deliver babies in cases where this would have been impossible years ago. This same technology has allowed infants to survive who formerly would have perished before the advent of neonatal intensive care units. Withdrawal of life support continues to be controversial. These and other problems have caused us to discuss the rationing of medical care on the basis of cost.

Identifying the challenges seems to come easy for all of us. The solutions are more elusive. The responsibility rests squarely on our shoulders because there is no other group that can perform the duties of our specialty. The answers will not come easily, but competence, compassion, caring, and consideration are qualities that we must demonstrate. It is within this spirit that we need to work with other members of our society in reaching solutions.

The fact that our members prepare and present original articles during this meeting is evidence of a commitment to communicate and a hunger to acquire knowledge. Continued education is a trademark of good medical care. Attendance at society meetings is an indication of a desire to provide a more enlightened level of care for our patients. This is commendable.

We have passed through a period of cynicism when no one respected doctors, clergymen, or any other professions. Our patients have been identified as "clients," and we have been labeled "vendors." I, as probably you, find these labels to be very offensive, but with the passing of time we have come to realize that change must be met with understanding rather than "knee-jerk" reaction. When most of us entered practice, there existed a strong physician-patient bond that was based on trust and respect, especially in obstetrics. The majority of young fathers today seem to enjoy being with their wives during the childbirth experience. Many advocates felt that so-called "bonding" would reduce the distressingly high divorce rate, but that has not proved to be the case. How can we make a difference?

Most of us have come from families that have given us strength and support. We are well educated and motivated to carry out our jobs to the best of our ability. It has been and still is our privilege to be physicians, and it remains our responsibility to care for all members of our society, regardless of ability to pay. We have made sacrifices to attain our professional status; however, we must continue to sacrifice ourselves if we are adequately to understand and care for our patients' needs.

In our age of sophisticated technology, we must remember to be compassionate. Dr. Charles Bryan[1] in the April 1990 issue of *The South Carolina Medical Journal* recalled an incident during his residency. The patient was a frail, elderly woman with acute myocardial infarction. She was going into shock, and he called a cardiologist. Entering the intensive care unit, having never seen the patient before, the cardiologist abruptly announced: "Mrs. Jones, I'm Dr. Smith, and I'm going to put this catheter in your heart." Dr. Bryan went on to state that many persons would criticize Dr. Smith on the grounds that this approach to the patient epitomized our profession's present-day shortcomings, namely, that we have made great strides in technical proficiency but along the way have lost our human touch. However, he goes on to defend "Dr. Smith" in that our first obligation to our patients is to perform competently that which we do.

At one extreme those reimbursed primarily for procedures do their patients a disservice to the extent to which "compassion" is omitted from the competent performance of those procedures. At the other extreme, those reimbursed primarily for rendering compassion (such as pastoral care-givers) do patients a disservice when competent technology is sacrificed. The truly wonderful thing about medicine is that most of us can do both. But in striking a balance we should always strive to do well that which we are actually paid to do.

Compassion alone is not sufficient. We must have: (1) courage (which makes everything possible), (2) competence (which establishes credibility), (3) consistency (which ensures that competence will become reality), and (4) compassion. However, we should be aware that "compassion without competence at what one claims to be able to do is fraud; consistent application of competence is compassion."

Let us be positive. The secret of caring for the patient is to be able to do something for the patient and to do it well. Never before has it been possible to do so much for so many.[2]

Yes, it is our role as physicians to keep pace but never forget the patient in the provision of health care. We will not be able to fill all the needs and we must recognize that, unfortunately, we will be confronted with situations that are out of our control.

And, yes, it is the personal application of our skills that has made our profession what it is today, and it is continued care and concern that will allow us to be judged fairly.

In closing, may we remember what a friend reminded me—"that a patient doesn't care how much we know, but she must know how much we care."

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2. Hester LL Jr. The pursuit of excellence. Am J Obstet Gynecol 1965;93:301-5.

Characterization of in vitro chemosensitivity of perioperative human ovarian malignancies by adenosine triphosphate chemosensitivity assay

Robert T. Gerhardt, MPH, James P. Perras, PhD, Bernd-Uwe Sevin, MD, PhD, Edgar Petru, MD, Reinaldo Ramos, BS, Lucy Guerra, and Hervy E. Averette, MD
Miami, Florida

We report the in vitro chemosensitivity of primary and recurrent human ovarian tumor samples analyzed by adenosine triphosphate chemosensitivity assay. We defined sensitivity as a ≥70% decrease in intracellular adenosine triphosphate versus control at 20% of the reported peak plasma concentration per agent tested. Twenty of 21 assays (95.24%) were completed successfully. Single-agent and combined dose-response patterns consisting of decreasing viability with increasing drug concentration were observed consistently. Thirteen primary tumors were assayed, with 15.4% sensitive to cisplatin, 7.7% sensitive to 4-hydroxycyclophosphamide and 53.8% sensitive to their combination. Seven recurrent tumors were assayed, with 14.3% sensitive to cisplatin, 28.6% sensitive to 5-fluorouracil, and 42.9% sensitive to their combination. Dose-response characteristics and in vitro sensitivity rates reported in this article are consistent with reports of patient response in the literature. We conclude that adenosine triphosphate chemosensitivity assay is an efficient and reliable instrument for the in vitro chemosensitivity assessment of human tumors and warrants further clinical investigation. (Am J Obstet Gynecol 1991;165:245-55.)

Key words: Adenosine triphosphate chemosensitivity assay, bioluminescence, chemosensitivity, ovarian cancer

Researchers and clinical oncologists have long recognized the potential value of pretreatment assessment of chemosensitivity for patients with cancer.[1] Efforts in this direction to date have produced mixed results.[2] The issues of in vitro evaluability, heterogeneity of tumor subpopulations,[2] emerging drug resistance,[4] and the questionable role of clonogenic or stem cells in malignancy have provided formidable obstacles to the development of an in vitro assay possessing in vivo predictive value. Assays such as the human clonogenic assay, succinate dehydrogenase inhibition, and radioactive thymidine uptake index are often inconclusive, and they possess a high rate of nonevaluability.[5-7] Consequently, in vitro chemosensitivity testing to date has remained primarily within the purview of the basic scientist, leaving the clinician to select chemotherapeutic regimens on an empiric basis.

Ovarian malignancy is a formidable disease for the patient and a challenge for the clinician. Its insidious onset with relatively late detection, high malignant potential, tendency toward early metastasis, and rapid development of resistance to chemotherapy contribute to a generally poor prognosis for the afflicted patient.[8] The standard treatment for both primary and recurrent malignancy includes cytoreductive surgery followed by adjuvant chemotherapy with one or a combination of several agents, commonly including cisplatin and cyclosphosphamide. 5-Fluorouracil (5-FU) is sometimes used in the salvage therapy of recurrent malignancies. Prognosis is related to clinical stage, histologic grade, tumor type, the amount of unresectable residual tumor at the time of cytoreductive surgery, and response to postoperative adjuvant chemotherapy.

The adenosine triphosphate (ATP) chemosensitivity assay has been reported as an efficient, highly evaluable, and cost-effective means of characterizing the in vitro chemosensitivity of cell lines and fresh human tumors, with promising preliminary clinical correlations.[9-11] With ATP extraction by acid cytolysis and measurement by luciferin-luciferase bioluminometry, ATP chemosensitivity assay assesses chemotherapeutic-induced cell kill of the entire tumor sample (including proliferative and nonproliferative subpopulations) by comparing total ATP concentration in treated samples versus untreated controls. Total ATP concentration as

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assessed by ATP chemosensitivity assay demonstrates a strong correlation with in vitro viable cell count ($R^2 > 0.95$).[9-11]

This study was designed to assess critically the use of the ATP chemosensitivity assay as an in vitro model of human tumor chemosensitivity. Both primary and recurrent human ovarian carcinomas obtained intraoperatively were analyzed over a wider range of chemotherapeutic dosages than in prior studies to more accurately characterize chemosensitivity and the related phenomena of drug resistance, heterogeneity, and single- versus combined-agent effects. In addition, in vitro chemosensitivity rates obtained by ATP chemosensitivity assay were compared with clinical reports of patient response to identical single agents and combinations to determine whether the in vitro results were consistent with clinical experience.

Material and methods

Tumor tissue specimens were obtained from 21 patients who underwent exploratory laparotomy for surgical staging and cytoreduction of ovarian carcinoma at the University of Miami/Jackson Memorial Medical Center between Feb. 12, 1988, and Nov. 31, 1989. Primary ovarian carcinoma was diagnosed in 14 of these patients whereas recurrent ovarian cancer was diagnosed in seven. The biopsy specimens were obtained under sterile conditions from fresh tumors immediately after resection. Samples were subjected to ATP chemosensitivity assay, which is described in detail in a prior publication.[10] Briefly, ATP chemosensitivity assay begins with mechanical disaggregation followed by gentle enzymatic disaggregation, which yields a suspension of single cells and small clusters of up to 30 cells each. The cell suspension is then plated over a soft agar underlayer in standard 24-well culture plates. The agar underlayer allows growth and proliferation of neoplastic cells, but impedes the survival of nonneoplastic tissues by preventing anchorage. Varying concentrations of individual chemotherapeutic agents and combinations are then applied to the wells in triplicate. After a 6-day incubation period, intracellular ATP is extracted by trichloroacetic acid cytolysis, followed by buffering of the resultant solution and luminometry using a luciferin-luciferase enzyme complex. This complex generates quantifiable light energy when bound to ATP molecules. The average luminosity of drug-treated wells versus controls is then compared to characterize the cytotoxic effect of the drug or combination in question.

Primary tumor samples were tested for sensitivity to cisplatin, 4-hydroxycyclophosphamide (an active metabolite of cyclophosphamide) and to a combination of these two agents; recurrent tumor samples were tested for sensitivity to cisplatin, 5-FU, and to their combination. All chemotherapeutic agents and combinations were tested at final peak plasma concentration equivalents[12] of 10%, 20%, 50%, 100%, and 500% after taking into consideration a final volume composed of cell suspension, agar underlayer, and the aliquot of chemotherapeutic agent. The drug concentration calculations were based on the following 100% peak plasma concentration values reported in micrograms of agent per milliliter of plasma: 2.5 μg/ml of cisplatin, 6 μg/ml of 4-hydroxycyclophosphamide, and 50 μg/ml of 5-FU. The value for 4-hydroxycyclophosphamide represents 20% of the reported peak plasma concentration of cyclophosphamide (30 μg/ml); this reflects the resultant plasma level of this metabolite after its intrahepatic conversion.

After obtaining the bioluminescence counts and calculating the ATP concentration in each control and treated culture well, average ATP concentration and coefficient of variance were calculated for each control and treatment type–dose group. The difference in average ATP concentration between treatment and control groups was then calculated. Dose-response curves were constructed from these data. In this study, sensitivity to a chemotherapeutic agent or combination was defined as a ≥70% average reduction in ATP concentration versus control at a dose of 20% of the peak plasma concentration for the agent or combination. If a chemotherapeutically treated tumor sample failed to meet these criteria it was designated resistant to that respective agent or combination.

The concentrations of chemotherapeutic agents necessary to produce a 50% decrease of cellular ATP (IC_{50}) were calculated by median effect analysis. This method uses linear regression of the log of drug concentration versus the log of the quotient of the fraction of affected cells divided by the fraction of unaffected cells [Log (C) versus $\text{Log}(f_a/f_u)$] to produce a least-squares equation from which IC_{50} may be calculated.[13] These calculations were also used to determine the type of combined drug response pattern.

Results

During this study, 21 subject tumor tissue specimens eligible at time of surgery underwent ATP chemosensitivity assay. Twenty assays were completed successfully, yielding an evaluability rate of 95.24%. One assay displayed evidence of bacterial contamination and was excluded from the study. Among the triplicate control wells studied, coefficients of variation ranged from 2.3% to 15.9%. Sixty-five percent of all triplicate controls analyzed possessed coefficients of variation of ≤10%, whereas 95% possessed coefficients of variation of ≤15%. Greater variation was noted among chemotherapeutically treated triplicates, with coefficients of variation at 20% peak plasma concentration drug con-

Table I. Fifty percent inhibitory chemotherapeutic concentration (IC_{50}) for primary human ovarian carcinomas treated with cisplatin, 4-hydroxycyclophosphamide, and their combination

Tumor	*IC_{50} DDP*	*IC_{50} 4HC*	*IC_{50} Combined*
Ag-1	12.73	16.98	8.51
Cah-1	109.82	9.87	5.15
Cer-1	461.88	22.85	NA*
Fr-1	59.94	29.08	0.74
Prov-1	75.15	70.51	NA*
Ric-1	273.53	56.23	NA*
Rol-1	21.30	30.70	NA*
Wei-1	46.29	51.66	NA*
Wes-1	180.22	160.33	NA*
HG-1	21.04	21.12	13.60
HC-1	42.46	12.95	6.24
IS-1	17.30	10.62	4.80
GL-1	26.60	19.77	17.0
Average IC_{50}	103.7	39.43	8.01*
SD	131.7	40.93	5.58*
R^2 (DDP vs 4-HC)	0.05		

DDP, Cisplatin; *4HC,* 4-hydroxycyclophosphamide. IC_{50} is reported as percentage of peak plasma concentration.

*Because of a change in laboratory procedure while this study was still in progress, combined drug concentrations for these tumors were prepared only for 10%/10%, 20%/20%, 50%/20%, and 100%/10% of cisplatin and 4-hydroxycyclophosphamide, respectively. The lack of three consecutive equivalent drug concentrations rendered it impossible to calculate IC_{50} values, thus the combination statistics were calculated without them.

Table II. Fifty percent inhibitory chemotherapeutic concentration (IC_{50}) for recurrent human ovarian carcinomas treated with cisplatin, 5-FU, and their combination

Tumor	*IC_{50} DDP*	*IC_{50} 5-FU*	*IC_{50} Combined*
MC-1	30.91	42.48	21.59
MS-1	0.0004	8.88	0.0004
PO-1	39.92	0.013	8.49
LP-1	15.38	11.19	3.13
MR-1	9.93	12.41	8.64
La-1	45.88	38.27	20.07
YK-1	62.90	25.97	21.89
Average IC_{50}	27.5	19.88	11.97
SD	23.72	15.98	9.13
R^2 (DDP vs 5-FU)	0.45		

DDP, Cisplatin. IC_{50} is reported as percentage of peak plasma concentration.

centration ranging from 6.01% to 67.19%. Among these triplicates, 40% possessed coefficients of variation of <10%, 72% possessed coefficients of variation of <20%, and 90% possessed coefficients of variation of ≤30%.

Table I presents the IC_{50} for cisplatin, 4-hydroxycyclophosphamide, and their combination for all primary tumors analyzed in this study. Table II presents the IC_{50} for cisplatin, 5-FU, and their combination for all recurrent tumors analyzed in this study. The differential effects of single agents and their combination in the treatment of individual tumors may be assessed by comparison of the IC_{50} values; these effects are so noted. In addition, statistics are provided in each table relating to the IC_{50} distribution for each agent and combination, as well as for the coefficient of correlation between the individual agents. One will note on inspection of Table I that combined agent IC_{50} calculations are available for only seven of the 13 primary tumors. Because of a change in laboratory procedure while data collection for this study was still in progress, combined drug concentration assays for six of the 13 primary tumors included in this study were prepared in a manner that departed from the original protocol. The lack of three consecutive equivalent drug concentrations rendered it impossible to calculate IC_{50} values for these six tumors; thus the primary tumor combination chemotherapy statistics were calculated on the basis of data available for the remaining seven tumor samples only.

Tables III and IV present the comparative chemosensitivity rates of primary and recurrent tumors for single agents and combinations as assessed by ATP che-

Table III. In vitro response of primary ovarian carcinomas compared with clinical response reported in the literature

Chemotherapeutic agent or combination	*% Sensitive by ATP-chemosensitivity assay*	*Reported clinical response (complete + partial)*
Cisplatin	15.4%	6%[23] to 72%[16]
4-Hydroxy-cyclophosphamide	7.7%	5%[21] to 65%[25]
Cisplatin and 4-hydroxycyclophosphamide	53.8%	52.4%[20] to 72.0%[16]

Total tumors evaluated: 13.

Table IV. In vitro response of recurrent ovarian carcinomas compared with clinical response reported in the literature

Chemotherapeutic agent or combination	*% Sensitive by ATP-chemosensitivity assay*	*Reported clinical response (complete + partial)*
Cisplatin	14.3%	20%[15] to 72%[16]
5-FU	28.6%	9%[26] to 58%[27]
Cisplatin and 5-FU	42.9%	NA

Total tumors evaluated: 7.

mosensitivity assay versus the clinical response to these same agents and combinations as reported in the literature.

Fig. 1, *A*, *B*, and *C*, depicts the dose-response curves of the 13 primary ovarian carcinomas to cisplatin, 4-hydroxycyclophosphamide, and their combination, respectively. The vertical axis represents the percentage of intracellular ATP at each drug concentration versus control, whereas the horizontal axis represents the respective drug concentration as percentage of peak plasma concentration. Note the great variation of individual tumor responses and the general trend toward decreasing intracellular ATP as the concentration of chemotherapeutic agent rises.

Fig. 2, *A*, *B*, and *C*, likewise depicts the dose-response curves of the seven recurrent ovarian carcinomas to cisplatin, 5-FU, and their combination, respectively. Graph features are similar to those described for Fig. 1, as are the observations made regarding individual tumor variation and general dose-response character.

Fig. 3 depicts the response of primary ovarian tumors to cisplatin, 4-hydroxycyclophosphamide, and their combination at drug doses of 20% of the peak plasma concentration. The vertical axis represents the percentage of intracellular ATP versus control, whereas the horizontal axis depicts the different drug treatment types. The horizontal line marks the point of 70% reduction in intracellular ATP. Note the heterogeneity of response to each individual drug and the trend toward greater sensitivity to the combined regimen.

Fig. 4 depicts the response of recurrent ovarian tumors to cisplatin, 5-FU, and their combination at drug doses of 20% of the peak plasma concentration. Graph features are similar to those described for Fig. 3. Note the heterogeneity of response to each individual drug and the trend toward greater sensitivity to the combined regimen.

Fig. 5 consists of four graphs that illustrate the types of single- versus combined-agent dose-response patterns of tumors analyzed in this study. For each graph, the vertical axis represents the percentage of intracellular ATP versus control, whereas the horizontal axis depicts the concentration of the chemotherapeutic agents tested. Note the general dose-response character of each graph, with a trend toward decreasing viability as measured by intracellular ATP as drug concentration rises. The horizontal line marks the point of 70% intracellular ATP versus control.

Fig. 5, *A*, depicts an antagonistic profile, with resistance to cisplatin, sensitivity with a strong dose-response to 5-FU, and sensitivity with a less-pronounced dose-response to their combination. There appears to be a decrease in cytotoxicity of the combination (or antagonism of action) because of cisplatin. This observation is consistent with the IC_{50} calculations reported for this tumor (PO-1) in Table II. Fig. 5, *B*, depicts a subadditive profile in which resistance to cisplatin, sensitivity, a strong dose-response to 4-hydroxycyclophosphamide, and a nominal increase in sensitivity to combined therapy is observed. Fig. 5, *C*, depicts an additive profile in which one notes sensitivity and a more linear dose-response to both single agents and their combination. The combined-agent curve indicates the possibility of a greater sensitivity than to either single agent. Fig. 5, *D*, depicts a synergistic profile that has

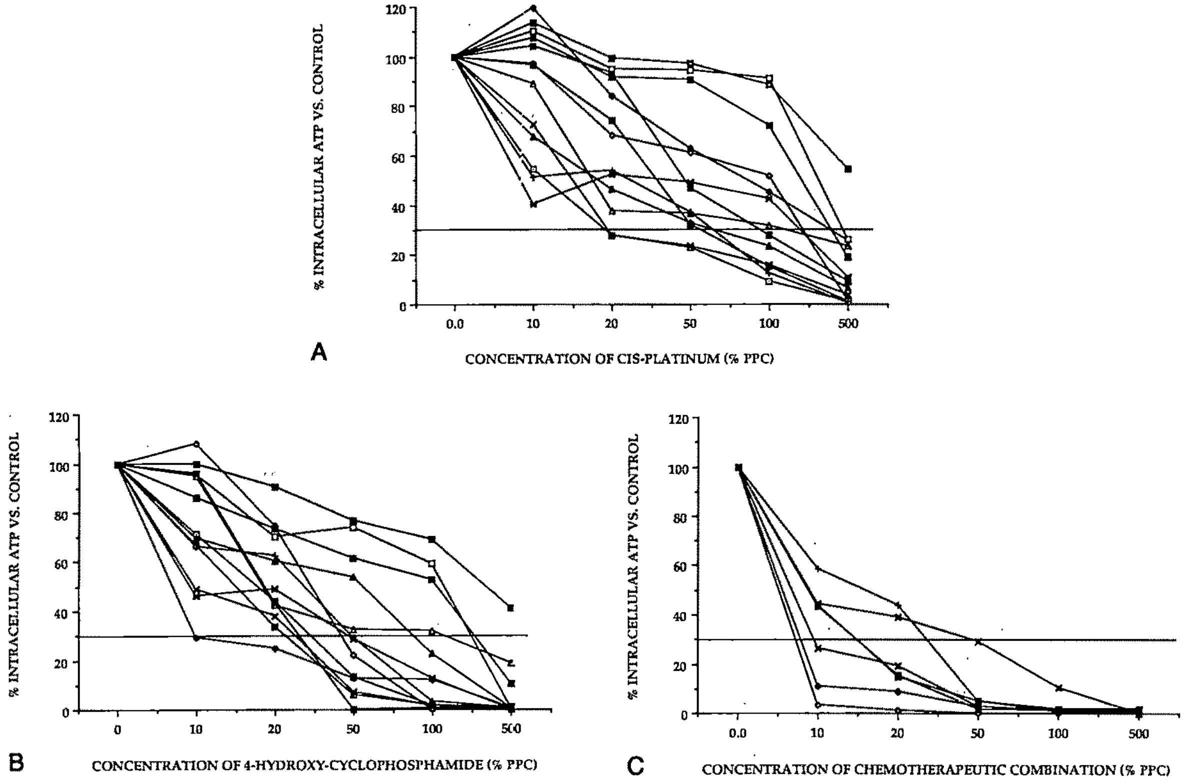

Fig. 1. In vitro chemosensitivity profiles of primary ovarian carcinomas to cisplatin, 4-hydroxycyclophosphamide, and their combination. Dose-response curves of 13 primary human epithelial ovarian carcinomas are depicted. On each graph, the *vertical axis* represents ATP concentration in chemotherapeutically treated samples as a percentage of controls. The *horizontal axis* represents the specific concentrations of each chemotherapeutic agent and combination tested, as a percentage of peak plasma concentration *(PPC)*. The *horizontal line* at 30% intracellular ATP versus control is provided as a marker of this study's criterion for sensitivity. **A**, Depicts the dose-response of the 13 primary tumors to cisplatin; **B**, depicts the dose-response to 4-hydroxycyclophosphamide (an active metabolite of cyclophosphamide); **C**, depicts the dose-response to combined exposure with both chemotherapeutic agents.

been observed on rare occasion. Resistance to both single agents up to the highest drug concentrations is noted, whereas the combined curve shows strong sensitivity at the lowest drug doses.

Comment

ATP chemosensitivity assay is an experimental method for efficiently and reliably predicting in vivo chemosensitivity on the basis of in vitro data. As developed by Perras and Sevin at the University of Miami, ATP chemosensitivity assay uses standard tissue culture materials and facilities, may be performed within a period of 6 days, and requires minimal training of the investigator beyond standard tissue culture method. With incorporation of an agar underlayer to retard growth of nonneoplastic cells, ATP chemosensitivity assay measures the in vitro chemosensitivity of human tumor suspensions representing viable cells in all stages of the growth cycle, not merely those cells that are actively proliferating. The effect of single-agent and combination chemotherapeutic treatment on tumor suspensions is assessed in terms of decreased ATP concentration present in treated wells versus controls, implying a differential in viability as a result of chemotherapeutic treatment. Preliminary clinical studies indicate a high level of predictive accuracy for the ATP chemosensitivity assay, evidenced by a strong correlation between predicted chemotherapeutic results and clinical response.[10]

Previously, we reported the chemosensitivity profiles of 22 ovarian tumor samples collected intraoperatively and exposed to chemotherapeutic concentrations of 20% and 100% peak plasma concentration, respectively.[10] The present study was designed to elucidate further the response patterns of fresh human ovarian malignancies to a wider range of chemotherapeutic doses and drug combinations. Comparison of in vitro sensitivity rates to clinical reports of patient chemotherapeutic response as found in the literature was also planned.

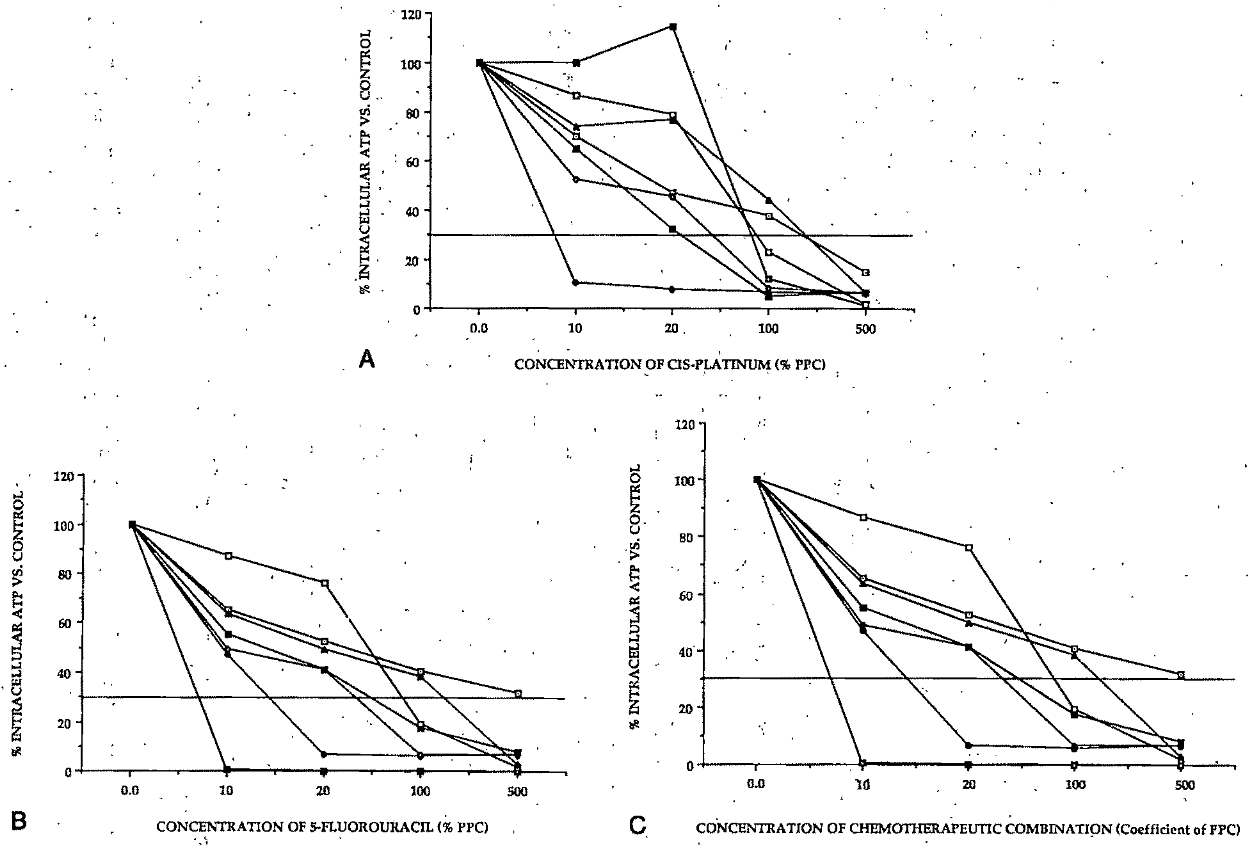

Fig. 2. In vitro chemosensitivity profiles of recurrent ovarian carcinomas to cis-platinum, 5-FU, and their combination. Dose-response curves of seven recurrent human epithelial ovarian carcinomas are depicted. On each graph, the *vertical axis* represents ATP concentration in chemotherapeutically treated samples as a percentage of controls. The *horizontal axis* represents the specific concentrations of each chemotherapeutic agent and combination tested, as a percentage of peak plasma concentration *(PPC)* as reported in the text. The *horizontal line* at 30% intracellular ATP versus control is provided as a marker of this study's criterion for sensitivity. **A,** Depicts the dose-response of the seven recurrent tumors to cisplatin; **B,** depicts the dose-response to 5-FU; **C,** depicts the dose-response to combined exposure with both chemotherapeutic agents.

Consistent with prior experiences at this facility, we observed a 95.24% evaluability rate in ATP chemosensitivity assays performed throughout this study. This success rate is significantly better than those observed with other popular chemosensitivity assays such as the human tumor stem cell assay, which possesses an evaluability rate of 40% to 70%.[2]

We selected chemotherapeutic agent concentrations on the basis of coefficients of the reported peak plasma concentrations (micrograms per milliliter) for each respective single agent after intravenous administration.[12] Peak plasma concentrations of combination treatments were based on the mixture of equivalent peak plasma concentration multiples of each of the individual agents used. The wide range (10% to 500%) and the number of data points (five) were selected to minimize interpolation and extrapolation in dose-response calculations and to provide data for subsequent correlative studies designed to determine which concentration might yield the strongest clinical predictive value. The selection of 500% peak plasma concentration also would serve a potential use because of the clinical application of intraperitoneal chemotherapeutic administration using higher doses of selected agents,[14] and would be of value in defining drug resistance.

Our decision to use a chemotherapeutic agent concentration of 20% peak plasma concentration as the in vitro predictor of in vivo tumor chemosensitivity was made on the basis of empiric findings performed at this facility. An explanation of this rationale rests with the practical estimation of a chemotherapeutic agent concentration within tumor tissue of roughly 20% peak plasma concentration resulting from the maximum possible intravenous dosage (100% peak plasma concentration). Continuous exposure to a chemotherapeutic agent at 20% peak plasma concentration is a close approximation of the area under the curve of dose versus time exposure of in vivo intravenous treatment,

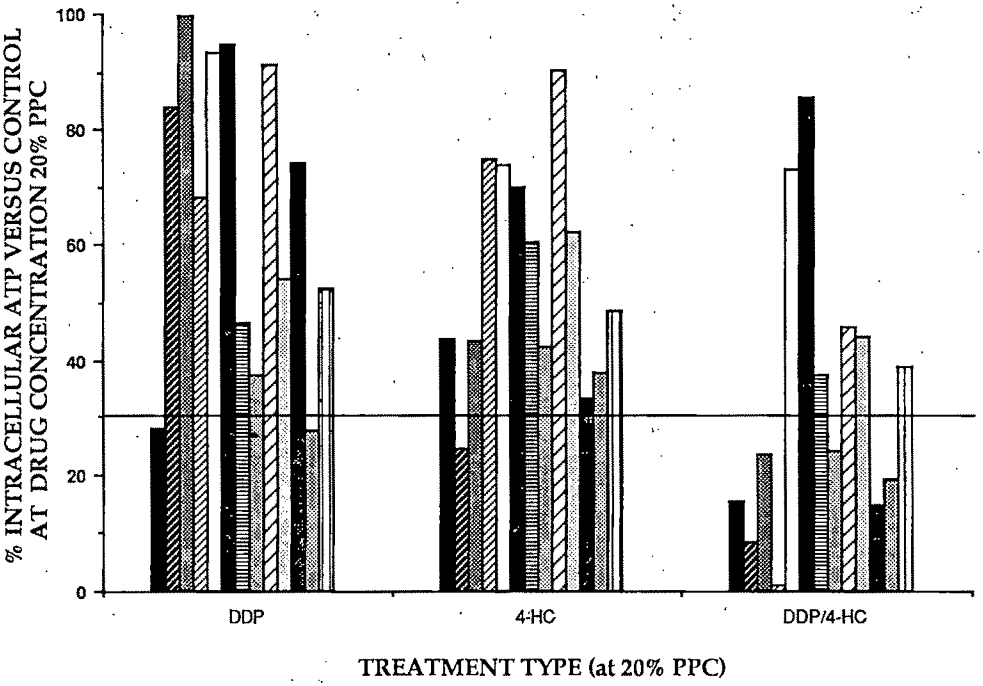

Fig. 3. In vitro response of primary human ovarian carcinomas to cisplatin, 4-hydroxycyclophosphamide, and their combination. The in vitro response of 13 primary ovarian tumors to chemotherapeutic agents at 20% peak plasma concentration *(PPC)* is depicted. The *vertical axis* represents ATP concentration in chemotherapeutically treated samples as a percentage of controls. The *horizontal axis* depicts each of three drug-treatment subgroups (*DDP,* cisplatin; *4-HC,* 4-hydroxycyclophosphamide; *DDP/4-HC,* combination therapy). The three subgroups depict the response of the 13 tumors to each individual agent and to their combination, respectively. Each tumor is represented by the same bar in each of the three subgroups. The *horizontal line* at 30% intracellular ATP versus control is provided as a marker of this study's criterion for sensitivity.

although this may vary among specific drugs and combinations.

The characterization of the chemosensitivity patterns of single and combined agents yielded interesting results. With regard to cisplatin, 15.4% of primary tumors were sensitive, whereas 14.3% of recurrent tumors were sensitive by this study's criteria. Cisplatin IC_{50}s were widely distributed for both primary (mean IC_{50}, 104% peak plasma concentration; SD, 131%) and recurrent tumors (mean IC_{50}, 29% peak plasma concentration; SD, 22%), which is consistent with the wide variation in patients' responses to this agent as observed clinically. When the two groups were compared, no significant difference was detected ($p < 0.16$). We conclude from these analyses that there is no consistent pattern of emerging drug resistance between primary and salvage treatment with cisplatin; on this basis, we infer that failure of primary cisplatin treatment would not preclude its use in second-line therapeutic regimens. This observation is consistent with clinical reports of successful cisplatin salvage therapy after its failure as a primary treatment.[15, 16]

Primary tumor response to 4-hydroxycyclophosphamide contrasted with the primary tumor response to cisplatin in several respects. Of 13 primary tumors tested, a smaller percentage (7.7%) showed sensitivity to 4-hydroxycyclophosphamide than to cisplatin (15.4%) by our criteria. Whereas wide variations were again evident, we observed a mean IC_{50} for 4-hydroxycyclophosphamide (39% peak plasma concentration; SD, 40%) that was lower than that of cisplatin (104% peak plasma concentration; SD, 131%), and that approached significance ($p < 0.11$). It is also worth noting that the deviation around the mean IC_{50} of 4-hydroxycyclophosphamide was less than that of cisplatin, indicating a somewhat greater consistency of response in this study population.

Chemotherapeutic regimens that include cisplatin and 5-FU are sometimes used in the salvage chemotherapy of recurrent ovarian carcinoma.[14-17] Generally, the response to salvage therapy is significantly less dramatic than the original therapeutic response; in the clinical environment, such therapy seldom succeeds. Of the recurrent tumors subjected to ATP chemosensitivity assay in this study, 14.3% were sensitive to cisplatin, 28.6% were sensitive to 5-FU, and 42.9% were sensitive to their combination. The observation that some recurrent tumors were sensitive to these chemotherapeu-

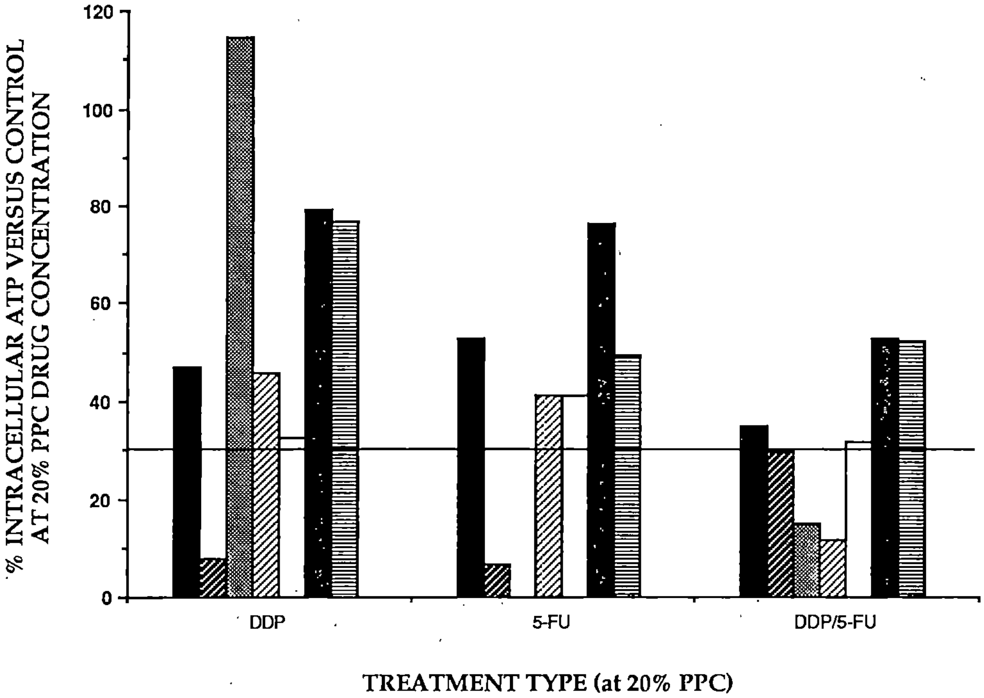

Fig. 4. In vitro response of recurrent human ovarian carcinomas to cisplatin, 5-FU, and their combination. The in vitro response of seven recurrent ovarian tumors to chemotherapeutic agents at 20% peak plasma concentration *(PPC)* is depicted. The *vertical axis* represents ATP concentration in chemotherapeutically treated samples as a percentage of controls. The *horizontal axis* depicts each of three drug-treatment subgroups (*DDP*, cisplatin; 5-FU, *DDP/5-FU*, combination therapy). The three subgroups depict the response of the seven tumors to each individual agent and to their combination, respectively. Each tumor is represented by the same bar in each of the three subgroups. The *horizontal line* at 30% intracellular ATP versus control is provided as a marker of this study's criterion for sensitivity.

tic agents shows the potential for successful salvage treatment provided that an effective agent or combination can be found. We believe this point underscores the immense potential value of the ATP chemosensitivity assay for the rapid assessment and determination of specific chemosensitivity profiles of residual or recurrent tumors on second-look laparotomy, which may differ significantly from the chemosensitivity profiles of the primary tumor. Indeed, the greatest value of ATP chemosensitivity assay may be shown in the future by its use as a means of directing salvage therapy. Here, time constraints and disease advancement necessitate the expeditious selection and undertaking of specific treatment. In such situations, ATP chemosensitivity assay could be used in the rapid screening of numerous cytotoxic agents and combinations to uncover those demonstrating sensitivity to a specific patient's recurrent malignancy.

Chemotherapy with combined agents has been shown to be more efficacious than single-agent treatment in the clinical environment.[17-20] Tables I and II show the general trend toward lower IC_{50}s for combination chemotherapy than for the constituent single agents in tumors analyzed by ATP chemosensitivity assay. Among the 13 primary tumors from which seven combined cisplatin–4-hydroxycyclophosphamide IC_{50}s were available, the mean IC_{50} for combination therapy (8% peak plasma concentration; SD, 5.6%) was lower than that of cisplatin (103.7% peak plasma concentration; SD, 131.7%), and closely approximated significance ($p < 0.075$). The mean IC_{50} for combination therapy was also lower than that of 4-hydroxycyclophosphamide (39.4% peak plasma concentration; SD, 41%); this difference closely approximated significance as well ($p < 0.062$). The seven recurrent tumors analyzed bore similar, although not as profound results: the mean IC_{50} for combination therapy was lower than that of cisplatin ($p < 0.081$); however, combined therapy did not approach significance when compared with 5-FU ($p < 0.28$). Tables III and IV show that 53.8% of primary tumors and 42.9% of recurrent tumors were sensitive to combined chemotherapeutic treatment by this study's criteria, often in the face of resistance to the same agents when tested individually. These findings are consistent with the generally accepted principle that combined anticancer chemotherapy is more effective

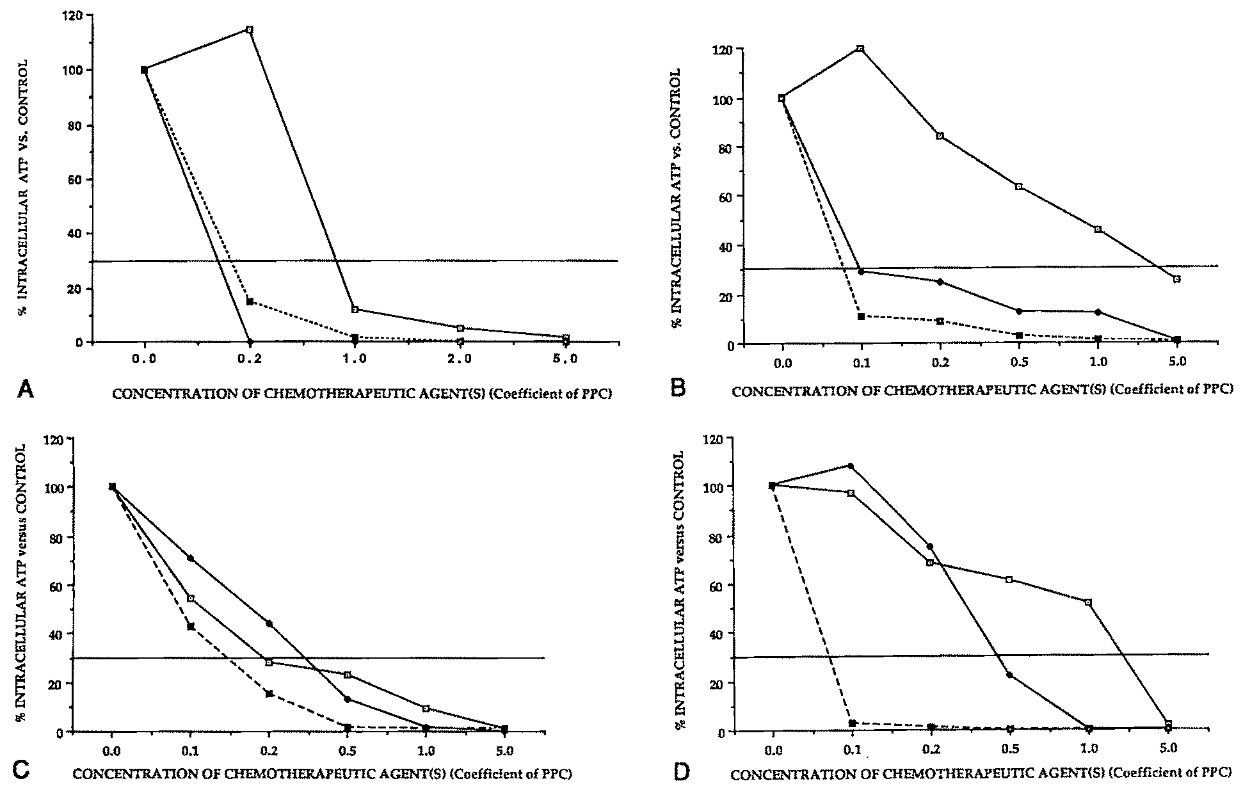

Fig. 5. In vitro dose-response profiles of individual human ovarian carcinomas to single-agent and combined chemotherapy. Dose-response curves of four representative epithelial ovarian carcinomas are depicted, showing response to two single agents and the effect of their combination on each tumor. The *vertical axis* represents ATP concentration in chemotherapeutically treated samples as a percentage of controls. The *horizontal axis* represents the specific concentrations of each chemotherapeutic agent and combination tested, as a percentage of peak plasma concentration *(PPC)*. The *horizontal line* at 30% intracellular ATP versus control is provided as a marker of this study's criterion for sensitivity. *Solid lines* represent the dose-response to single agents (*open square*, cisplatin; *solid diamond*, 4-hydroxycyclophosphamide or 5-FU, respectively), whereas the *dotted line* represents dose-response to the combination of both agents (*solid square*, combined agents). **A**, Depicts the response of a recurrent tumor sample to cisplatin, 5-FU, and to their combination, reflecting an antagonistic combined-agent profile. **B**, Depicts the response of a primary tumor sample to cisplatin, 4-hydroxycyclophosphamide, and to their combination, with a subadditive profile. **C**, Depicts the response of a primary tumor sample to cisplatin, 4-hydroxycyclophosphamide, and to their combination, with an additive profile. **D**, Depicts the response of a primary tumor sample to cisplatin, 4-hydroxycyclophosphamide, and to their combination, with a synergistic profile.

than single-agent treatment. It is also submitted as further evidence of the consistency between ATP chemosensitivity assay and clinical experience.

Tumor heterogeneity is a challenging issue in oncology.[3] The presence of unique aneuploid subpopulations within a single tumor mass has been documented by deoxyribonucleic acid (DNA) flow cytometry, whereas histochemical studies and other biochemical methods have been used to detect physiologic differences among subpopulations within a single tumor mass.[3, 21, 22] These pathophysiologic changes, most notably the acquisition of drug resistance, are clinically evident in persons in whom resistance to an agent develops during multiple treatment courses. Heterogeneity of response among histologically identical tumors obtained from different patients is also observed. This phenomenon is evidenced by populations of patients with histologically identical tumors with identical staging who respond differently to treatment with the same chemotherapeutic agent or combination.

Many of the tumors analyzed during this study showed evidence of heterogeneity as described in this article. Once again, the reader's attention is directed to Tables I and II, where wide variations in IC_{50}s are noted for different tumors treated with the same agent or combination, as well as among the different single agents tested on each individual tumor. Figs. 1 and 2 display this phenomenon graphically. In general, dose-response relationships were observed in terms of decreasing concentrations of intracellular ATP as chemotherapeutic dosage increased. The patterns of response among idividual cases, however, varied considerably. On one extreme, some tumors showed virtually complete cell kill at low chemotherapeutic doses, whereas other tumors possessed surviving fractions at drug concentrations of 500% peak plasma concentration, which would be well above the maximum intravenous concentration that could be attained in a human system. These observations show quantitatively the heterogeneity and drug resistance characteristics of malignant cell populations. They also underscore the potential value of characterizing individual patients' chemosensitivity profiles before selecting treatment.

After examining and interpreting the in vitro data, the next question we considered was whether ATP chemosensitivity assay possessed clinical relevance. Toward this end, we reviewed the literature with regard to reports of clinical response. With the use of the 70% reduction in intracellular ATP versus control at a drug concentration of 20% of the peak plasma concentration as the criterion for sensitivity, we compared the percentage of tumors sensitive to drugs by this criterion to clinical reports. Tables III and IV show these comparisons. Concerning the response of primary tumors, the in vitro drug sensitivity rates observed for cisplatin, 4-hydroxycyclophosphamide, and their combination fell within the rather wide bracket of clinically reported rates of drug response. Also, it is worth noting that the sensitivity rate to combined therapy was greater than that for either individual drug.

With regard to the response of recurrent tumors, one notes that the in vitro sensitivity rate of 5-FU fell within the bracket of clinically reported rates of drug response. The in vitro sensitivity rate of tumors to cisplatin as reported in this study was slightly lower than that of clinical reports; this may be explained in part by the limited size of our study population (seven tumors); the same may be said for the clinical reports, each of which cites fewer than 25 patients. At present, no data are published with regard to clinical response to combined cisplatin and 5-FU. Also note that the sensitivity of assayed tumors to combined therapy was greater than that reported for each individual agent. Whereas only seven recurrent tumors were evaluated in this study, the findings are consistent when compared with clinically observed responses.

At the conclusion of this study, several important hypotheses were confirmed concerning the applicability of the ATP chemosensitivity assay to human tumor chemosensitivity assessment. First, fresh human tumor evaluability rates of >95% have been obtained consistently by ATP chemosensitivity assay, confirming that it is a highly efficient instrument for the chemosensitivity assessment of intraoperative human tumor tissue specimens and is reliable in terms of evaluability. Second, ATP chemosensitivity assay dose-response patterns for tumors tested were consistent with clinical observations; namely, the assay consistently detects decreasing tumor cell viability as chemotherapeutic agent concentration rises, and it quantitatively shows that combined-agent cytotoxicity is generally equal to or greater than that observed for single agents. Further, the rates of single- and combined-agent drug sensitivity by ATP chemosensitivity assay are consistent with clinical response rates as reported in the literature, indicating that the ATP chemosensitivity assay may prove to be a reliable model for the prediction of clinical response. These observations lead us to conclude that the ATP chemosensitivity assay warrants further investigation of its predictive value in the clinical environment to determine its value as a tool in directed postoperative adjuvant chemotherapy.

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Single-center comparison of results of 1000 prenatal diagnoses with chorionic villus sampling and 1000 diagnoses with amniocentesis

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Large multicenter studies have confirmed the safety and accuracy of chorionic villus sampling as a prenatal genetic diagnostic procedure, but there have been few single-center evaluations. We report our experience with 1000 consecutive chorionic villus sampling procedures compared with 1000 consecutive amniocentesis procedures during the same period. The procedures were performed by the same genetic counselors, sonographers, obstetricians, and laboratory personnel. Indications for referral, demographic characteristics of patients, numbers of attempts per patient, fetal loss rates, laboratory results, and evaluation of accuracy are included. Analysis of all data suggests that chorionic villus sampling is a safe and accurate alternative to amniocentensis in our community-based teaching hospital. (AM J OBSTET GYNECOL 1991;165:255-63.)

Key words: Chorionic villus sampling, amniocentesis, prenatal diagnosis, genetic counseling

Prenatal genetic diagnosis by amniocentesis has been used successfully for >25 years. The development of chorionic villus sampling (CVS) in the last 5 years has presented an important option for patients. Because large collaborative studies have been conducted at major medical centers in the United States[1] and Canada,[2]

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Table I. Indications for prenatal diagnosis

	No. of CVS procedures	*No. of amniocentesis procedures*
AMA (≥35 at EDD)	855	545
AMA, high MSAFP	0	13
AMA, low MSAFP	0	66
High MSAFP	0	134
Low MSAFP	0	110
Neural tube defect, before	0	24
Neural tube defect, family history	0	7
Aneuploidy, before	47	13
Aneuploidy, family history	25	6
Ultrasonographic abnormality	0	29
Single-gene disorder	22	3
Parental translocation	15	5
Multiple spontaneous abortion	2	11
Parental anxiety	20	8
Teratogen	0	9
Other	14	17
TOTAL	1000	1000

AMA, Advanced maternal age; *EDD,* estimated date of delivery; *MSAFP,* maternal serum α-fetoprotein.

there is little information as to procedural complication rate, safety, and accuracy of CVS in community-based medical centers. We report our experience with 1000 consecutive CVS procedures compared with 1000 consecutive amniocentesis procedures during the same period performed by the same genetic counselors, sonographers, obstetricians, and laboratory personnel.

Material and methods

During the period from September 1985 to July 1989, 1000 CVS procedures and >1000 amniocenteses were performed in the Department of Obstetrics and Gynecology, University of South Carolina School of Medicine. Initially, our patient population came from within a 500-mile radius because ours was the only facility in the southeastern United States offering CVS. By mid-1988, multiple centers began offering CVS; our catchment area has since decreased to a more appropriate 50- to 100-mile radius for both CVS and amniocentesis referrals.

Before each prenatal diagnosis, a genetic counselor met with the patient to record a family history, discuss general procedure, review the advantages and limitations of testing, and obtain informed consent. Patients seen early in gestation were given the option of CVS or amniocentesis. The same genetic counselors, obstetricians, cytogenetic technologists, and geneticists served both data groups.

CVS was performed as previously reported.[3] Transabdominal real-time ultrasonography was performed with a scanner (Ultramark IV, Advanced Technology Laboratories, Inc., Bothell, Wash.) and a 3.5 MHz transducer. Fetal gestational age was determined by fetal crown-rump length measurement according to the method of Robinson and Fleming.[4] All CVS procedures were carried out by one of two experienced obstetricians (R.V.W. or C.F.S.). Maternal serum α-fetoprotein value tests and midtrimester ultrasonography were suggested as routine follow-up for patients having CVS.

To compare CVS with amniocentesis, we gathered data on 1000 consecutive patients having amniocentesis during the period May 1987 to June 1989. The timing in this data group differs because approximately twice as many amniocenteses are performed in our center as CVS. Amniocenteses were performed as previously described.[5]

For the first 700 CVS studies, the direct method of chromosome analysis was used exclusively.[6] The average turnaround time for CVS direct studies was 4.2 days. Cultured analyses were completed in an average of 17 days. Amniocytes were cultured by the flask method.[7] Turnaround time for amniotic fluid cytogenetics averaged 17 days. If CVS or amniocyte studies showed mosaicism, an abnormal finding, or questionable results, additional preparations were analyzed.

Amniocentesis follow-up information was gathered retrospectively. Referring physicians were asked to review their delivery notes to provide information on pregnancy outcome. We did not contact patients in this group and therefore lack data on side effects of the amniocentesis. Postprocedural complications, as reported by our first 1000 patients having amniocentesis studied in 1976 to 1982, were compared with those of the first 1000 patients with CVS (unpublished data). Since the advent of our program, follow-up data for patients who had had CVS have been collected prospectively by sending a letter to the referring obstetrician on the expected delivery date requesting specific information such as complications of delivery, birth weight, and information regarding possible congenital malformations. Letters sent to patients who had had CVS were timed to arrive 6 weeks after the delivery date to seek information regarding complications of the procedure, delivery, growth of the baby, and comments about prenatal diagnosis.

Reports of fetal loss from each data group were reviewed and classified as early (≤4 weeks after procedure), late (>4 weeks after procedure), and remote (≥26 weeks' gestation). Every effort was made to establish the underlying mechanism of each fetal death.

Table II. Single-gene disorder indications and results

Indication	CVS		Amniocentesis	
	No. studied	*No. affected*	*No. studied*	*No. affected*
Cystic fibrosis	5	2	1	1
Sickle cell disease	5	0	2	1
Duchenne-type muscular dystrophy	4	1		
Fabry's disease	2	0		
Ornithine transcarbamoylase deficiency	2	1		
GM_1 gangliosidosis	1	0		
Hemophilia (factor VIII deficiency)	1	0		
Hunter's syndrome and Duchenne-type muscular dystrophy	1	0		
Metachromatic leukodystrophy	1	0		
TOTAL	22		3	

Table III. Demographic characteristics of study population

	CVS		Amniocentesis	
	No.	%	*No.*	%
Maternal age (yr, median)	36		35	
Race				
White	902		605	
Black	65		375	
Asian, other	13		11	
Not recorded	20		9	
TOTAL	1000		1000	
Education				
<12 yr	7	1.9	66	11.2
High school	71	18.9	213	36.2
Some college	58	18.1	118	20.0
College degree	230	61.2	192	32.6
TOTAL	376		589	
Income				
<$10,000	5	1.4	69	13.9
$10-$19,999	20	5.7	102	20.5
$20-$40,000	105	29.7	176	35.4
>$40,000	223	63.2	150	30.2
TOTAL	353		497	

Results

Indications for prenatal diagnosis for both CVS and amniocentesis are shown in Table I. Table II lists the specific single-gene disorder for which diagnoses were sought and the number of affected pregnancies. Demographic characteristics of the study population are reflected in Table III. Patients optionally provided information on their educational background and total family income, accounting for $N < 1000$ in these tables. Ninety-three percent of CVS patients underwent the procedure at 9 to 11 weeks' gestation, with 5.1% at 7 to 8 weeks and 1.2% at 12 weeks. Eighty-seven percent of patients had amniocentesis at 16 to 20 weeks, with 5.2% <16 weeks, 4.8% at 20 to 23 weeks, and 2.1% >23 weeks.

The number of CVS catheter insertions are shown in Table IV. Overall, 79% of CVS procedures were successful at first attempt. Comparatively, 99.3% of amniocenteses were successful with one needle insertion, and none required three attempts. CVS procedural attempts analyzed chronologically by hundreds show that in the first 400 studies approximately 70% of cases required one attempt. In the later stages of study, the first attempt was successful in 85% of cases. Our current practice is to stop sampling after two unsuccessful attempts.

Cytogenetic results of the 1000 CVS and 1000 amniocentesis studies are presented in Table V. No cases of chromosome, neural tube, or single-gene defects were missed by these studies. There were 22 CVS and 14 amniocenteses with aneuploid or ring chromosome results. All but one translocation were known to be familial; one balanced Robertsonian translocation found at amniocentesis remains unclassified because

Table IV. Attempts at chorionic villus sampling analyzed by operator experience

Patient sequence	No. of catheter insertions		
	1	*2*	*3*
1-100	71	22	7
101-200	68	25	7
201-300	68	23	9
301-400	74	15	11
401-500	84	12	4
501-600	85	11	4
601-700	85	14	1
701-800	82	16	2
801-900	88	10	2
901-1000	89	11	0
TOTAL	794	159	47

the parents declined testing. One CVS result was reported as being 46,XY on the basis of direct preparation only and was discovered to be incorrect at the birth of a phenotypically normal female infant with a 46,XX karyotype. The pregnancy involving the CVS ring chromosome result was electively terminated after the direct analysis showed >20 cells to be affected. The patient opted not to wait for results from the culture. The cultured villus and tissue analysis from products of conception showed no evidence of the ring chromosome in >80 cells.

Eleven (1.1%) CVS studies showed mosaic results. One of these pregnancies was lost before further studies could be undertaken. Of the remaining 10, nine resolved to a normal karyotypes at amniocentesis and were phenotypically normal at delivery. The CVS finding of 45,X/46,XY was confirmed by amniocentesis in a blood sample from the newborn. Two amniotic fluid samples yielded mosaic findings, both confirmed in the the newborn.

In 23 CVS studies no chromosome results were obtained. In 15 cases an inadequate sample was taken. In three cases the villi appeared to be hydropic; two of these pregnancies terminated in spontaneous abortion within weeks of sampling. In three cases an adequate sample failed to yield analyzable chromosomes for study. Twelve of these patients had normal amniocentesis results, one repeated the CVS with normal results, one amniocentesis yielded trisomy 18, and six patients declined further study. Ten amniotic fluid studies yielded no cytogenetic result. In two cases a bloody or clotted sample prevented adequate cell growth, and in two others the imminent death of the fetus was suspected to have negatively influenced cell growth.

Fetal loss relative to the number of CVS catheter insertions or amniocentesis needle insertions is shown in Table VI. The percentage of loss increased significantly with catheter insertion; a 12% loss rate occurred when three passes were required. Because >99% of amniocenteses were successful with a single attempt, there was little opportunity for procedure-related trauma.

Table V. Cytogenetic results

	No. of CVS procedures	*No. of amniocentesis procedures*
46,XY	485	500
46,XX	449	466
Trisomy 22	1	0
Trisomy 21	11	6
Trisomy 20	0	1
Trisomy 18	4	2
Trisomy 16	2	0
Trisomy 13	0	1
69,XXY	1	0
47,XXX	0	1
47,+mar	1	1
46,XY,r(6)	1	0
45,X	1	2
Balanced translocation	8	7
Unbalanced translocation	2	1
Mosaic	11	2
No result	23	10
TOTAL	1000	1000

Postprocedural complications are shown in Table VII. The majority of patients with two complications reported cramping and bleeding. Pregnancy outcomes after the prenatal diagnostic procedures are presented in Table VIII. Elective pregnancy terminations included all CVS pregnancies with unbalanced, nonmosaic karyotypes, four pregnancies affected with single gene disorders, and two pregnancies in which CVS yielded no result. Four patients in the CVS group electively terminated pregnancies after abnormalities were found later (anencephaly, gastroschisis, toxoplasmosis, and trisomy 18 syndrome). Eight cytogenetically normal pregnancies were terminated without stated reasons. In the amniocentesis group, nine aneuploid pregnancies and seven pregnancies with anomalies detected by ultrasonography were electively terminated.

The percentage of loss immediately after procedures appears to be almost exactly the same in the CVS and amniocentesis groups, whereas there are small differences in the >4 weeks after procedure and at ≥26 week's gestation intervals (Table VII). Approximately 96% of both groups resulted in live births, with birth weights (mean ± SEM) of 3429 (±19) gm and 3225 (±26) gm, respectively.

Comment

Recent collaborative studies from the United States and Canada have indicated that CVS is a safe, accurate modality when practiced under certain conditions.[1,2] Medical centers reporting CVS experience have gen-

Table VI. Fetal loss according to catheter or needle insertion

No. of insertions	CVS		Amniocentesis	
	No.	*%*	*No.*	*%*
1	25/794	3.1	36/993	3.6
2	9/159	5.7	0/7	0.0
3	6/47	12.8	0	0.0

Table VII. Postprocedural complications

Complication	CVS		Amniocentesis	
	No.	*%*	*No.*	*%*
Cramping	41	4.3	132	17.3
Bleeding	120	12.6	12	1.6
Fluid loss	0	0.0	9	1.2
Fever	3	0.3	0	0.0
Two postprocedural complications	23	2.4	10	1.3
No follow-up	44		235	

Table VIII. Pregnancy outcome

	CVS		Amniocentesis	
	No.	*%*	*No.*	*%*
Total studies	1000		1000	
No follow-up	25		155	
Elective termination	42		16	
Study population	933		829	
Loss < 4 wk after procedure	15	1.6	15	1.8
Loss > 4 wk after procedure	21	2.3	10	1.2
Loss ≥ 26 weeks' gestation	4	0.4	11	1.3
Live-birth pregnancy	894	95.8	793	95.7
Birth weight (gm)	3429 ± 19		3225 ± 26	

erally been large facilities with high patient volume. We were interested in exploring the safety and accuracy of CVS in a community-based teaching hospital and in comparing the advantages and disadvantages of CVS versus amniocentesis in such a setting.

The current study demonstrates a change in referral patterns, in that patients of advanced maternal age are opting more often for CVS than for amniocentesis. Indications for referral revealed that 86% of patients having CVS and 55% of patients having amniocentesis underwent prenatal testing for advanced maternal age indications; 84% of the first 1000 patients having amniocentesis at our center were indicated for age-related risk.[6] Changing referral patterns are also suggested in that 32% of our patients having amniocentesis were indicated for study because of an abnormal maternal serum α-fetoprotein value, an indication that barely existed just 5 years ago. Also of note, 9% of patients of advanced maternal age underwent amniocentesis after a high or low maternal serum α-fetoprotein result became a risk factor. The development of high-quality prenatal ultrasonography has also created an indication for testing in the second and third trimesters with the discovery of anomalies on ultrasonography. Patients with histories of aneuploidy, single-gene disorder, chromosome translocations, and anxiety opted more often for CVS than for amniocentesis, whereas patients at risk because of a history of spontaneous abortion more often chose amniocentesis, perhaps from concern over procedural safety.

The demographic differences between the two data groups were highly significant (race $\chi^2 = 277.05$, $df = 2$, $p < 0.01$; education $\chi^2 = 93.06$, $df = 3$, $p < 0.01$; income $\chi^2 = 121.79$, $df = 3$, $p < 0.01$). The amniocentesis population closely resembled the overall white to black birth ratio in South Carolina (60% to 40%); in contrast, the CVS group was 90% white, with more education and a higher average family income than the amniocentesis population. This perhaps reflects the trend that educated patients with higher incomes are the first to use new testing services and may reflect the general population more accurately as CVS becomes a standard procedure.

Our study clearly demonstrates that amniocentesis is almost always successful on the first attempt and that CVS often requires two attempts. Our CVS data and those of other authors support the concept that there is a short learning curve in performing CVS, with fewer attempts and better samples as operator and sonographer experience increases.[8, 9] We currently get adequate samples on our first attempt in 89% of cases (CVS patients 1001 through 1286).

Bleeding after CVS was the most frequently encountered postprocedural complication. Patients who had amniocentesis had cramping more frequently. Although three patients who had had CVS reported fever, only one CVS pregnancy was lost by septic abortion.

Our follow-up on pregnancy outcome in the patients who had CVS is more complete than the amniocentesis follow-up and is probably due to our active solicitation of information from patients who had CVS and their physicians within weeks of delivery. Elective pregnancy termination occurred more frequently in the CVS group, which included patients who were at higher risk of having abnormalities and patients who terminated their pregnancies for personal reasons, five of which were questionably related to sex selection.

The pregnancy loss rate ≤ 4 weeks after procedure was very similar in both data groups ($t = 0.299$;

$p = 0.3821$), suggesting that procedure-related risk is similar for CVS and amniocentesis. The >4-week post-procedure loss rate was slightly higher in the CVS group and may represent the normal loss rate at this gestational age. We are unable to explain the difference found at the later stage of pregnancy. The total loss rate in both groups was 4.3%.

The pregnancy outcomes of the first 719 of the 1000 patients having CVS presented in this study were described in detail in our previous report[3]; we found that maternal age, gravidity, quantity of villi, and gestational age apparently did not contribute to fetal loss. Chi-square analysis, however, revealed an increasing risk of fetal loss with multiple attempts at CVS ($\chi^2 = 14.8914$; $p = 0.0006$). The findings in our series are consistent with the increased risk of pregnancy loss associated with multiple catheter insertions. Birth weight was significantly larger ($t = 6.33$; $p << 0.001$) in the CVS group, as was also noted by Ferguson et al.[10] The larger birth weight may reflect differences in socioeconomic status or indications for referral.

Twenty-five years of experience has proved amniocentesis to be a highly accurate technique. The technique of CVS cytogenetic diagnosis and the estimate of its accuracy are still evolving. We began our program using only the direct method of chromosome analysis; referring physicians and patients were highly satisfied with the rapid diagnosis. As cultured CVS methods were developed to get better-quality chromosome preparations, reports of discrepancies between direct and cultured results suggested study of both. Practical limitations exist, however, in that a small villus sample might preclude both studies; the cost of two chromosome analyses increases the cost of CVS by at least 150%. Culture techniques also require a longer waiting period for results and are often reported as the patient begins the second trimester of pregnancy. The newly developed interphase cytogenetic techniques[11] might be applied to CVS, and for the direct identification of common trisomies and fetal gender might be applied to amniocentesis. This method would provide a rapid screen and, combined with a karyotyping technique, may offer an accurate, quicker, and less expensive analysis, particularly for the CVS patient who seeks first-trimester diagnosis.

Mosaic findings in CVS study will likely continue to be a problem in approximately 1% of cases. This may be the actual frequency of confined placental mosaicism in the normal population, given the consistency of its frequency across studies.[12, 13] Because most patients become anxious, they can be reassured that these findings are usually artifactual and generally resolved by amniocentesis.

Cytogenetic analyses of amniocytes rarely show pseudomosaicism or maternal contamination, whereas CVS studies often appear to be confounded by these findings. Although our experience with confined placental mosaicism is similar to that reported by other authors, we have not had the 18% to 27% rate of maternal cell contamination in cultured preparations that has been reported elsewhere.[14, 15] The incidence in our CVS group (10/1000) more closely approximates that of Hogge et al.[16] (3/600).

No false-negative or false-positive results from the CVS or amniocentesis series are known. CVS showed a ring chromosome in one pregnancy; that pregnancy was electively terminated on the basis of direct results only and may have represented a confined placental mosaicism. A CVS study incorrectly reported as 46,XY was, in retrospect, a case in which 12 cells from direct preparation yielded a clear 46 count in a patient at risk because of advanced maternal age. The morphology of the chromosomes was poor, however, and the study was miskaryotyped. These incidents serve to remind us that new technology must develop in an atmosphere of constant evaluation and investigation. When unusual findings occur, we must return to the older methods for verification or reevaluation. Patients and obstetricians like the speed of the direct chromosome study, but artifacts occur and chromosome shape may not be high resolution in nature.

Analysis of the data suggests CVS to be a safe and accurate alternative to amniocentesis in our center. Although it appears that patients at higher risk opt for the earlier CVS, amniocentesis referrals have increased since the early 1980s because of an increase in midtrimester screening by ultrasonography and maternal serum α-fetoprotein values. CVS will certainly not replace amniocentesis, particularly as our techniques for Down syndrome screening with maternal serum α-fetoprotein values, human chorionic gonadotropin, and other markers become more refined. Both CVS and amniocentesis will be used more frequently in the future as more genetic conditions become diagnosable via deoxyribonucleic acid methods. We are hopeful that with time CVS technology will be accepted as a safe, accurate, and effective obstetric procedure in the same way that prenatal diagnosis by amniocentesis has been accepted.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. GLENWARD T. KEENEY, Fayetteville, North Carolina (Official Guest). Since Ward et al.[1] and Simoni et al.[2] first established the clinical feasibility of CVS for fetal chromosomal analysis, there has been a concerted effort to ascertain the accuracy and the procedure-related risks of CVS compared with midtrimester amniocentesis. Both Canadian and U.S. collaborative studies on CVS reported no statistically significant difference in fetal loss rates between CVS and amniocentesis.[3-5] They did report a higher incidence of technical difficulties with CVS. Ferguson et al.[6] cited the variability in operator experience and the variability in the procedure and laboratory techniques as the main drawbacks of these trials. The series of Young et al. and of Hogge et al.,[7] Brambati et al.,[8] Ferguson et al.,[6] and Green et al.[9] appear to have a decided advantage in that each series involves a relatively large number of patients from a single center. All obstetric procedures were performed by one or two experienced operators using similar techniques of CVS sampling. Laboratory techniques were similar, clinically proved, and more consistent, with better trained and more experienced assistants.

Results obtained by these five groups of investigators appear to validate the statement of Ferguson et al.[6] Failure to make a cytogenetic diagnosis in patients who had CVS was more frequent in the Canadian trial and about equal between the U.S. study and the single-center groups.[4, 5] Maternal cell contamination of villus culture was two to three times more frequent in both collaborative series, and confined placental mosaicism was approximately equal in all the studies. Notwithstanding the rapid results obtained with direct trophoblast karyotyping, the general trend is to rely more on villus culture for accurate results.[10] Combining the direct method with villus culture optimizes quality and minimizes false-positive and false-negative findings,[11] but is is more costly and takes more time. According to Young et al., mosaicism will continue to be a problem in approximately 1% of patients having CVS.

In contrast to CVS, midtrimester amniocentesis is easier, requires fewer attempts, has a very low incidence of maternal cell contamination and mosaicism, and successfully yields a diagnosis 99% of the time.[3, 4, 6, 12] Postamniocentesis complications are also less frequent, with cramping being the most commonly reported symptom. Spotting and bleeding are seen rather frequently after CVS. In spite of these advantages a recent Swedish study revealed that midtrimester amniocentesis is perceived as a more "distressing experience" than CVS.[13] Whatever the inherent difficulties with CVS, it would seem that the technique has been reasonably perfected and that most of the technical problems have been solved. The data of Young et al. indicate a diagnostic accuracy of 97.7% for CVS, which compares quite favorably with a >99% accuracy for amniocentesis.

The safety issue, however, is more difficult to evaluate. Maternal infection is quite low with both methods, and fetal risk is the central issue. Absence of a control group in virtually all studies prohibits direct estimation of the total losses of the two techniques. Rhoads et al.,[3] who participated in the U.S. collaborative study, quote a 2.2% to 5.4% fetal loss rate after CVS in other published reports. In their own series they corrected for differences in gestational age and maternal age and concluded that the best estimate of excess risk for CVS over amniocentesis is 0.8%. The Canadian study gives a 0.6% excess loss for CVS. Green et al.[9] quote a 0.6% procedure-related fetal loss, but their study includes only those early losses in the first few weeks after procedure. Ferguson et al.[6] have a 2.4% loss after CVS and a 0.9% rate after amniocentesis up to 28 weeks. They caution, however, that the CVS loss rate does not take into account the "background fetal loss." This term refers to spontaneous losses between 9, 12, and 16 weeks in patients with normal first trimester ultrasonography and no invasive procedures.[3,6]

Table VIII in the study by Young et al. lists loss rates of 1.6% and 1.8% at 4 weeks after CVS and amniocentesis, respectively. They suggest that this may represent a procedure-related risk for the two procedures. Rates of late losses of >4 weeks but <12 weeks after procedure were 2.3% for CVS and 1.2% for amniocentesis, possibly representing "the normal loss rate at this gestational age." Presumably this is a reference to the background spontaneous fetal loss. Rates of remote losses >12 but <20 weeks after procedure were actually higher for amniocentesis (1.3%) than for CVS (0.4%). No explanation is available for this difference.

In an earlier article coauthored by Young et al.[14] and presented to the South Atlantic Association of Obstetricians and Gynecologists in 1989, data are presented on the first 719 CVS patients in the current study. The total corrected fetal loss from CVS are 4.1% in the earlier study, ≤26 weeks' gestation. The current study shows identical total-corrected fetal losses of 4.3% for CVS and amniocentesis. Although the statistics are confusing, virtually all investigators agree that CVS losses increase dramatically with the number of transcervical catheter insertions. Table VI demonstrates over a fourfold increase between one and three attempts.

What then is the procedure-related fetal loss for transcervical CVS? For midtrimester amniocentesis, the fetal risk is generally said to be 0.5%, perhaps as high as 1%.[15] Young et al. suggest a procedure-related loss in CVS of 1.6%, Green et al.[9] 0.6%, Rhoads et al.[3] 0.8%, and the Canadian series 0.6% in excess of the amniocentesis loss rate.[3,4] Transabdominal CVS, which has certain advantages over the transcervical method, has an equivalent degree of safety with the latter technique,[16,17] and controlled randomized trials are underway to compare the two CVS methods. Early amniocentesis at 10 to 14 weeks' gestation also shows considerable promise.[18] In an ongoing collaborative study by Buchanan et al. (personal communication) >3000 patients have been studied between 12 and 14 weeks with accurate cytogenetic diagnosis, minimal fetal losses, and accurate acetylcholinesterase determinations. The results on maternal serum α-fetoprotein determinations are also encouraging. The advantages of this technique are readily apparent.

It is abundantly clear that a more standardized method of reporting fetal loss rates is needed. The published statistics are often confusing, and comparisons of the relative safety of CVS with amniocentesis are frequently vague. If they are possible, prospective, controlled, randomized trials will be much more meaningful if fetal loss rates are reported in a uniform manner.

This criticism in no way detracts from the excellent work of Young et al. Their report is orderly and comprehensive and includes a large number of patients from a community-based hospital. The same experienced team performed the procedures throughout the study. Follow-up with the patients who had CVS is excellent, and there is little doubt of a future increased demand for CVS and amniocentesis. The data also support the conclusion of Young et al. that prenatal diagnosis by CVS at regional centers is a safe and accurate alternative to amniocentesis. Fetal loss, nevertheless, is probably somewhat greater with the former method.

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Discussion

Dr. William Kirkley, Fort Lauderdale, Florida. Did they do amniocentesis with those patients who had high maternal serum α-fetoprotein and normal level 3 ultrasonograms?

Dr. John W.C. Johnson, Gainesville, Florida. The 6% loss rate with two catheters seems high. Did the authors think about restricting their analysis to one-catheter studies?

Dr. Fay O. Redwine, Richmond, Virginia. Would the authors say what percentage of patients having CVS had to undergo amniocentesis—the total number—to improve the accuracy of their results or for other indications later in pregnancy.

Dr. Max C. Karrer, Jacksonville, Florida. I am a little confused over your statement of a 3% baseline loss rate when there is a time differential in comparing 4 weeks after CVS and 4 weeks after amniocentesis. How can you say that you have a 3% baseline loss rate?

Dr. Young (Closing). Amniocentesis was routinely offered to patients with elevated maternal serum α-fetoprotein values, regardless of normal-appearing ultrasonography findings. Recently we evaluated the patients with elevated maternal serum α-fetoprotein values in a portion of the amniocentesis study group.[1] Our findings support the use of ultrasonographic revised risks for open neural tube defects and allowing the patient to make an informed choice in reassessing the need for amniocentesis.

The 6% loss rate with two catheter insertions does seem high; however, this series represents our first 1000 CVS cases. The number of insertions has decreased with experience, and we are hopeful that the complication rate will, too. We will continue to monitor multiple sampling versus complications; if the loss rate remains at 5% to 6%, we should restrict sampling to one attempt.

Overall, 3.3% of patients having CVS had indications for amniocentesis because of failed CVS or mosaic results. Of these, three spontaneously aborted at <16 weeks, and six patients declined further study, yielding 2.4% of the CVS study group who also underwent amniocentesis.

The 3% baseline loss rate refers to spontaneous fetal loss at all gestational ages, not limited to 4 weeks after the procedure. One insertion was associated with a 3.1% loss rate in the CVS group and 3.6% rate in the amniocentesis group. This compares with the U.S. collaborative CVS study in which one insertion related to a total fetal loss rate of 2.9%.[2] This also compares with the National Amniocentesis Registry, which evidenced a 3.5% loss rate in the study group compared with a 3.2% rate in matched controls.[3]

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Quantification of cervical change: Relationship to preterm delivery in the multifetal gestation

Roger B. Newman, MD, Raleigh K. Godsey, MD, Janna M. Ellings, RN, CNM, Berry A. Campbell, MD, Daniel P. Eller, MD, and M. Clinton Miller III, PhD

Charleston, South Carolina

Prematurity is the major contributor to the very high perinatal morbidity and mortality associated with multifetal gestations. Antepartum cervical evaluation has been suggested as a way to better define the risk of preterm delivery in multifetal gestations. Weekly digital cervical examinations were performed in 86 twin and 7 triplet gestations that were being monitored in a special antepartum clinic. A cervical score was calculated from each examination by subtracting cervical dilatation in centimeters from cervical length in centimeters. Cervical scores decline gradually with advancing gestation and are influenced by parity and subsequent preterm delivery. Intervals until delivery decrease significantly with lower cervical scores. A cervical score ≤ 0 on or before 34 weeks' gestation was strongly predictive of preterm delivery (75%). Only 2 of 78 (2.6%) with a score >0 were delivered within 1 week of the examination. Cervical scoring is a simple, quantifiable, reproducible, and safe method of evaluating preterm delivery risk. An understanding of the prognostic significance of specific cervical scores may be of value in determining the need for obstetric intervention. (AM J OBSTET GYNECOL 1991;165:264-71.)

Key words: Cervical score, prematurity, multifetal gestations

Houlton et al.[1] suggested in 1982 that information obtained by antepartum cervical examination was effective in predicting the onset of both term and preterm labor in twin gestations. In 1988, Neilson et al.[2] reevaluated the predictive value of antepartum cervical assessment in a larger series of twin gestations. They determined that abnormality found at cervical examination before 34 weeks' gestation identified a group of twin pregnancies at extreme risk for preterm labor whereas a reassuring examination was rarely if ever associated with the spontaneous onset of labor within 1 week. Unfortunately, in spite of its large size, the study of Neilson et al. is difficult to translate into contemporary clinical practice. Their study group was composed entirely of African women in Zimbabwe who were all parous. In addition, their patients had gestational age assessment done primarily by Dubowitz scoring, relatively few antepartum visits, and no exposure to tocolytic therapy.[2]

The ability to better predict the onset of preterm labor would be of tremendous value in multifetal gestations because of the very high perinatal morbidity and mortality associated with these pregnancies. Prematurity is the major contributor to this increased perinatal morbidity and mortality.[3, 4] If antepartum cervical assessment can better define the risk of premature delivery in multifetal gestations, then it would be of great clinical value because of the simplicity, general applicability, and low cost of this intervention. For these reasons this study was undertaken to confirm the findings of both Neilson et al. and Houlton et al. regarding the prognostic capabilities of a quantifiable cervical score and to determine its role in the risk assessment of multifetal gestations.

Material and methods

In 1988, the Medical University of South Carolina established the Twins Clinic for women with multifetal gestations. It is staffed by a certified nurse-midwife and obstetric residents, and it is supervised by members of the maternal-fetal medicine section. Since its inception, 93 multifetal pregnancies (including 86 twin and 7 triplet gestations) have had follow-up to delivery.

The frequency of antepartum visits was established at intervals of every week or 2 weeks. At each clinic visit a digital examination was done to evaluate cervical dilatation in centimeters at the internal os, cervical length in centimeters, station of the presenting part, and consistency and position of the cervix. A cervical score was calculated as described by Houlton et al.[1] In brief, a cervical score is determined as follows: Cervical length (centimeters) − Cervical dilatation at internal os

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6/6/30069

(centimeters). For example, a cervix that is 2 cm long with a closed internal os gives a score of +2. A cervix that is 1 cm long with a cervix dilated 1 cm at the internal os gives a score of 0. A cervix that is 1 cm long with the internal os dilated 3 cm gives a score of −2.

Preterm birth was defined as delivery before 37 completed weeks of gestation. Preterm births included in this analysis were those occurring as secondary consequences of the spontaneous onset of premature labor or preterm premature rupture of the membranes. Fifteen patients were excluded from further analysis because of elective premature delivery. Indications for these elective premature deliveries include preeclampsia ($n = 6$), fetal growth abnormalities ($n = 4$), fetal distress ($n = 2$), documented fetal maturity ($n = 2$), and elective delivery in a case of monoamniotic, monochorionic placentation ($n = 1$). Both spontaneous and elective term deliveries (≥37 weeks' gestation) are included in this analysis. The cervical score data that follow are therefore derived from 71 twin and 7 triplet gestations.

Gestational age assessment was based on the estimated date of confinement as predicted by the first day of the last menstrual period, if confirmed by ultrasonography. When there was a discrepancy of ≥3 weeks between menstrual and ultrasonographic dating criteria, the estimated date of confinement was recalculated on the basis of the ultrasonographic findings.

Thirty-nine women received tocolytics for therapy of spontaneous preterm labor for an average duration of 19.4 ± 14.3 days. In four of these women tocolysis was successful, with a gestational age ≥37 weeks' gestation achieved, and for purposes of this analysis these are considered as term deliveries. Of the remaining patients receiving tocolytic therapy, tocolysis failed in three within 24 hours after therapy was instituted and 18 were delivered after a recurrent episode of spontaneous preterm labor. Nine women experienced preterm premature rupture of the membranes at a later date and 5 were delivered early for maternal or fetal indications.

Results are expressed as mean ± standard deviation. Analysis of the data was performed by the χ^2 test with Yates' correction, the Fisher exact test, analysis of variance for repeated measures, and the correlation coefficient. Significance was established at $p = 0.05$.

Results

The demographic characteristics of the 71 twin and 7 triplet gestations are included in Table I. Forty-four of the multifetal gestations (56%) were delivered preterm; 30 of these 44 were delivered after spontaneous preterm labor and 14 after preterm premature rupture of the membranes. The remainder (44%) were delivered at term after spontaneous labor ($n = 16$), after spontaneous rupture of the membranes ($n = 5$), or by elective induction of labor or cesarean section ($n = 13$).

Table I. Demographic characteristics of the twin ($n = 71$) and triplet ($n = 7$) gestations included in the cervical score analysis

Age (yr, mean ± SD)	25.3 ± 5.0
Race (No.)	
White	33 (42%)
Black	45 (58%)
Parity (No.)	
Nulliparous	20 (26%)
Parous	58 (74%)
Gestational age at diagnosis (wk, mean ± SD)	19.0 ± 7.0
Gestational age at initial cervical examination (wk, mean ± SD)	24.2 ± 4.0
Gestational age at delivery (wk, mean ± SD)	35.8 ± 3.2
Prenatal visits (No., mean ± SD)	9.0 ± 3.8
Cervical examinations (No., mean ± SD)	7.8 ± 3.8

There is a significant decrease in the mean cervical score with advancing gestation ($p \leq 0.001$) between 20 and 36 weeks' gestation, as demonstrated in Fig. 1. No racial differences could be identified in the cervical score data; however, Fig. 2 reveals that parous women have lower mean cervical scores, especially in later gestation, as compared with those of nulliparous women.

Table II provides the mean duration from the first appearance of any specific cervical score until delivery. The lower the mean cervical score, the shorter the time interval until delivery. The regression equation for this relationship is: Time until labor = 5.12 + 1.51 (Cervical score). The regression coefficient is significant at $p \leq 0.0001$ and the R^2 (percent of variation accounted for) is 91.3%.

Pregnancies complicated by preterm delivery also had consistently lower mean cervical scores with advancing gestational age as compared with those of women delivered at term (Fig. 3). The first appearance of a cervical score ≤0 correlated best with the gestational age at delivery ($R^2 = 0.54$). Table III presents the sensitivity, specificity, predictive values, and relative risk of preterm birth associated with a cervical score of ≤0 at or before 34 weeks' gestation. Table III also provides this analysis with respect to gestational age (20 to 28 and 29 to 34 weeks) and parity (nulliparous and parous).

Of particular note is that only 2 of 78 (2.6%) women experienced spontaneous preterm labor or preterm premature rupture of the membranes within 1 week of a cervical score >0. Preterm premature rupture of the membranes occurred in a triplet gestation at 34 weeks' gestation within 1 week of a cervical score of +1.5. Another patient with a twin gestation experienced

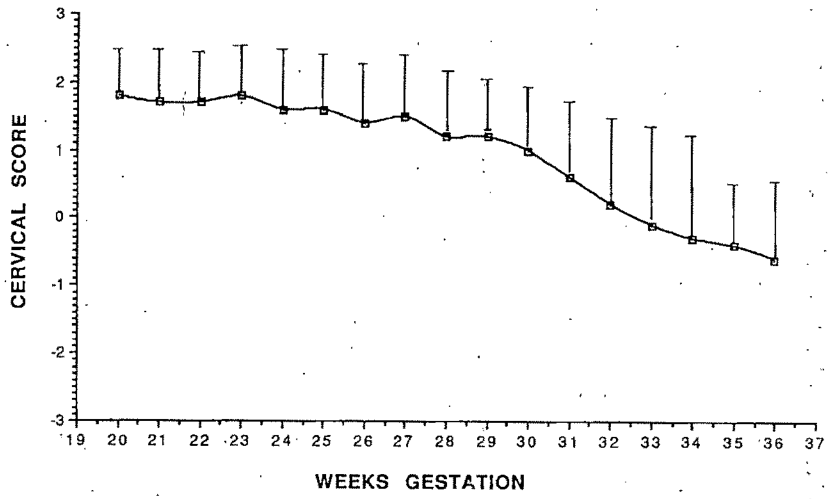

Fig. 1. Cervical score (mean ± SD) from 20 to 36 weeks' gestation for all multifetal gestations included in data analysis (N = 78).

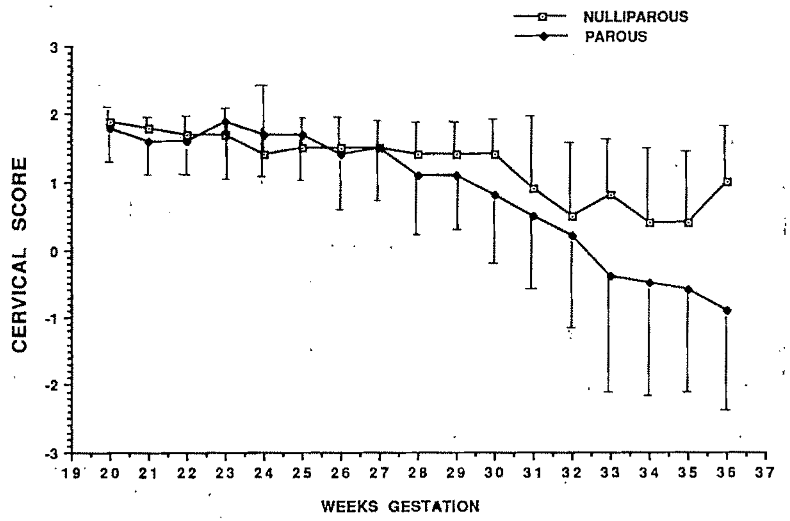

Fig. 2. Cervical score (mean ± SD) from 20 to 36 weeks' gestation for nulliparous (⊡, n = 20) and parous (▲, n = 58) multifetal gestations.

spontaneous preterm labor at 36 weeks' gestation within 1 week of a cervical score of +0.5.

Comment

Weekly cervical assessment in a group of women with multifetal gestations reveals a progressive decrease in the cervical score most notable after 30 weeks' gestation. As the cervical score decreases, the mean time until delivery shortens, and its absolute value correlates strongly with the timing of delivery. The time interval until delivery as associated with any specific cervical score in this study is very similar to the findings of Neilson et al. in their study of parous African women with twins. These similarities were found in spite of the fairly extensive use of activity restriction and tocolytic therapy in this study.

The high rate of preterm birth in multifetal gestations is a result of increased uterine activity presumably caused by myometrial overdistention.[5, 6] Cervical ripening can occur independently of uterine contractions as it is known that both endocrine factors and prostaglandins can alter cervical consistency; however, it is clear that prelabor uterine activity plays a major role in cervical dilatation and effacement. Uterine contractions initially cause progressive effacement, then later dilatation of the cervix, as cervical tissue is pulled upward, over the presenting part, and is incorporated into the lower uterine segment. It is believed by many that

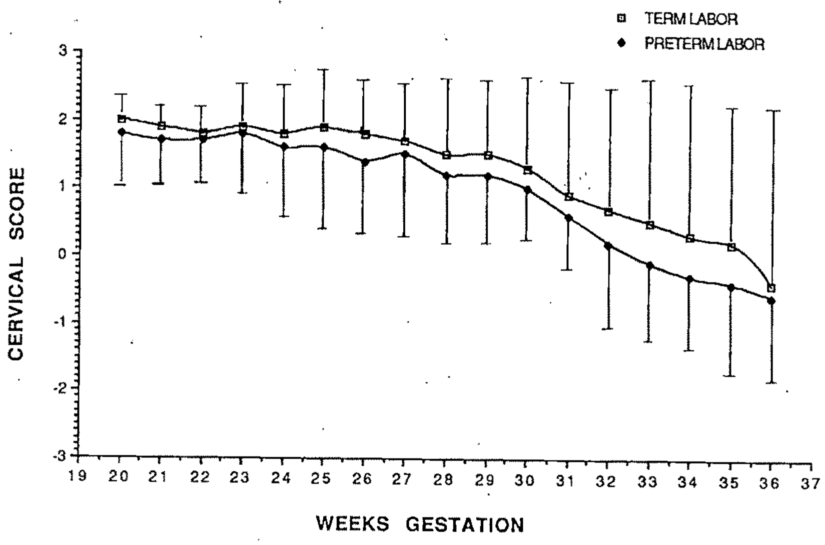

Fig. 3. Cervical score (mean ± SD) from 20 to 36 weeks' gestation for multifetal gestations with term (▫, $n = 34$) and preterm (♦, $n = 44$) delivery.

the frequency of these so-called Braxton Hicks contractions is related to the timing of the onset of labor.[7-9] The progressive fall in cervical score with advancing gestation appears to mirror the crescendo of prelabor uterine activity seen with multifetal gestations.[10]

The data presented suggest that information derived from a simple antepartum cervical examination can better define any patient's individual risk of preterm labor and delivery. Selecting a cervical score of ≤0 on or before 34 weeks' gestation as being abnormal was associated with a positive predictive value of 75% and a fourfold increased relative risk of preterm delivery. In the study of Neilson et al. a similar positive predictive value for preterm delivery was obtained with a cervical score of ≤ −2 or before 34 weeks' gestation.[2] It appears that in their population of parous African women a more advanced degree of cervical dilatation and effacement can be tolerated as compared with those of the patient population presented in this investigation.

We did identify that the earlier in gestation a cervical score of ≤0 is detected, the greater the positive predictive value that can be ascribed to it (i.e., 92% at 20 to 28 weeks' gestation). Parous women in this study tended to have lower mean cervical scores than did nulliparous women; however, this did not seem to significantly alter either the positive predictive value (72% vs 83%) or the relative risk (3.3 vs 4.3) of preterm delivery. As the cervical score becomes more negative at earlier gestational ages, the positive predictive value for preterm delivery approaches 100% but the sensitivity decreases because of the relative rarity of such abnormal antepartum cervical examinations. Obstetricians need complete knowledge of the evolution of cervical change in multifetal gestations to understand the prognostic significance of any specific cervical score.

Table II. Length of time from first appearance of specific cervical score to onset of labor

Cervical score	*No. of patients*	*Length of time to labor (wk, mean ± SD)*
+2.0	66	8.8 ± 4.2
+1.0	34	7.0 ± 3.5
+0.5	21	6.3 ± 2.9
0	25	3.7 ± 1.7
−0.5	18	3.9 ± 2.3
−1.0	26	3.2 ± 2.3
−2.0	14	2.0 ± 1.3
−3.0	12	1.5 ± 0.8

It seems most reasonable to use a cervical score of ≤0 as a marker of abnormal cervical status and increased preterm delivery risk. The first appearance of a cervical score ≤0 had the best correlation with the gestational age at delivery and provided the best sensitivity, specificity, and predictive values for preterm delivery. A cervical score of ≤0 seems to strike the correct balance between the number of women identified as being at increased risk of preterm delivery and the actual number of jeopardized babies correctly identified. The strength of the association between abnormal antepartum cervical scores and preterm delivery possibly may have been greater had it not been for the extensive use of tocolytic drugs. However, while these drugs delayed delivery for an average of almost 3 weeks, they allowed for the achievement of a term gestation in only four cases. Again, the similarity of our findings and those obtained by Neilson et al. in a pop-

Table III. Sensitivity, specificity, predictive values, and relative risk of premature delivery (<37 weeks' gestation) on basis of cervical score ≤0 on or before 34 weeks' gestation

Patient group	*Sensitivity*	*Specificity*	*Positive predictive value*	*Negative predictive value*	*Relative risk**	*p Value*
All patients	88%	62%	75%	81%	3.9 ($1.8<RR<8.7$)	≤0.0001
Patients 20-28 wk	33%	96%	92%	61%	2.0 ($1.4<RR<2.9$)	≤0.01
Patients 29-34 wk	87%	81%	73%	81%	3.8 ($1.7<RR<8.5$)	≤0.0001
Nulliparous	83%	75%	83%	75%	3.3 ($1.0<RR<11.4$)	≤0.02
Parous	91%	58%	72%	83%	4.3 ($1.5<RR<12.4$)	≤0.001

**RR*, Relative risk (with 95% confidence limits).

ulation not exposed to tocolytic therapy suggests a relatively insignificant role for these agents.

Of particular interest is the fact that only two women experienced spontaneous preterm labor or preterm premature rupture of the membranes within 1 week of having a cervical score >0. In the series of Neilson et al.[2] of 223 twins, none were delivered within a week of a cervical score ≥0. Women who can maintain a cervical score of >0 would seem to be good candidates for continued conservative observation rather than being subjected to any of the controversial obstetric interventions available to us. Several studies have failed to demonstrate any advantage to routine hospitalization and bed rest for either twin[11, 12] or triplet[13] gestations. Also called into question is the value of any degree of activity restriction in multifetal gestations short of hospitalization and bed rest. The routine use of prophylactic tocolytic therapy has not been clearly shown to be effective despite extensive use.[14, 15] Home uterine activity monitoring has been effective in making the early diagnosis of preterm labor in multifetal gestations but remains an expensive and labor-intensive approach to preterm birth prevention.[13, 16, 17] The differentiation of normal from abnormal cervical scores may be of value in designing clinical protocols or trials to determine the indications for and effectiveness of these obstretric interventions.

The cervical score was selected for evaluation because of its simplicity, quantifiability, reproducibility and focus on cervical dilatation and effacement. Among singleton gestations, changes in cervical dilatation and effacement are the best predictors of prematurity.[18-20] The previously described work of both Neilson et al. and Houlton et al. suggests that the cervical score has good prognostic value in multifetal gestations.[1, 2] At our own institution we have documented that interexaminer differences in the assessment of cervical dilatation and effacement are small, which suggests that the human hand is a sensitive instrument for examining these properties of the cervix.[18] The ability to quantify the cervical score allows for better communication between examiners and helps to eliminate subjective bias. Evaluations of cervical position and consistency are subjective by their very nature and provide little prognostic information once dilatation and effacement have been considered.[20] The station of the presenting part is significantly affected by parity, as well as by malpresentation, which is not uncommon in multifetal gestations.[1]

A final consideration is the safety of the antepartum cervical examination. Lenihan[21] has demonstrated increased risk of premature rupture of the membranes in women undergoing weekly cervical examination after 37 weeks' gestation. Bacterial colonization of the endocervix has been reported to be both increased and unchanged in patients with premature rupture of the membranes.[22, 23] However, no one has yet evaluated whether the antepartum cervical examination alters the endocervical flora.

Holbrook et al.[24] evaluated the role of the cervical examination as a component of the San Francisco preterm birth prevention program. On comparison with a large control group who did not receive antepartum cervical examination, there was no increase in rates of premature rupture of the membranes, chorioamnionitis, or endometritis, nor was there any relationship between the date of the last cervical examination and the ultimate onset of premature labor. Main et al.[25] described an inner-city population where the rates of premature rupture of the membranes were very high in a group of women who had routine antepartum cervical examinations, but the rate was also excessive among a high-risk control group who were not examined. Most important, successful local and regional programs preventing premature birth have all emphasized antepartum cervical examination.[26-28] The results of these investigations would make it seem unlikely that routine antepartum cervical examination is having any significant effect on rates of either spontaneous premature labor or preterm premature rupture of the membranes. In this study our rate of preterm premature rupture of the membranes was 15%, which is consistent with the rates reported in other reviews of multifetal gestations not having routine cervical examination.[29, 30]

Antepartum cervical examination in multifetal gestations for the calculation of a cervical score seems to be a safe, easily obtained, and valuable technique in refining our ability to predict the risk of preterm delivery. An understanding of the prognostic significance of specific quantifiable cervical data may be of great value in determining the need for obstetric intervention in multifetal gestations.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. JOHN B. WILLEY, Winchester, Virginia (Official Guest). When given the opportunity to prepare this review, I began looking for the earliest reference to the delivery of premature twins and I think I found it. From Genesis 25:24 and 25 we learn, "When her days to be delivered were fulfilled, behold, there were twins in her womb. The first came forth red, all his body like a hairy mantle; so they called his name Esau."

This to me is a fairly typical premature twin with the scalded appearance ("red" in the passage) and lanugo ("hairy mantle") that we have all come to recognize. We have no record of Rebekah's prenatal vaginal examinations.

Since Bishop[1] first looked to cervical evaluation for consideration of induction of labor, we have been made aware not only of the impression given by the vaginal examination but also of the quantification of such an examination. Recent studies such as that of Newman et al. have quite rightly deemphasized the more subjective elements of Bishop's score (consistency, station, and position) and concentrated on the more quantifiable cervical score obtained by subtraction of dilatation from effacement.

This study helped me to think more concretely about the importance of examination of preterm cervix. The data, while not falling as neatly as we might hope into categories of "at risk" and "not at risk," are impressive in several points. The relatively low risk for the patient with a cervical score >0 is helpful clinically. The increasing importance of a score <0 and the importance of its determination early in pregnancy, while intuitively apparent, are well documented here. The possibility that we can identify 73% of those twin gestations at risk of premature labor by a single examination before 34 weeks is intriguing.

One question I have concerning the study pertains to the reliability of multiple examiners to give like scores to one cervix. In spite of the authors' allusion to the small interexaminer differences in cervical assessment,[2] I remain somewhat skeptical. In my own hospital, where labor and delivery nurses perform many cervical examinations, I am often confronted with the differences between the examinations from nurse to nurse and from physician to physician. When I ask the nurse to tell me a patient's status, and she says, "Dr. X says she is 5 cm," both of us know that Dr. X's 5 and my 3 are equivalent. One of the strengths of the data in the work of Nielson et al.[3] from Zimbabwe, referred to in this article, is that all examinations were done by one of the two coauthors. A stronger case might be made if all examinations were done by one examiner, but I realize that the logistic problems of such a restriction are many.

A second question about this study is a fundamental problem with many medical surveys, that is, the need to react to the parameter under surveillance and thereby cloud the issue under study. One rather nice thing about the Zimbabwe article was that the data were unchanged by intervention and perhaps were more valid for that reason. The fact that tocolysis was so rare in the Zimbabwe study is a plus for that set of data that Dr. Newman and coworkers could not ethically achieve. I would like to know, in addition, what was done to each of these patients when a cervical change was documented. Such a discovery surely led to manipulations such as rest, tocolysis, and perhaps even cerclage or pessary use that hopefully would have altered the course of labor or prelabor cervical change and therefore affected the evolution of the cervical score. It is certainly possible to interpret the data as more valid because of these manipulations. As the authors pointed out, the association between abnormal antepartum cervical scores and preterm delivery may have been greater had it not been for the extensive use of tocolytic drugs.

Finally, I am troubled by the repeated vaginal examinations. Without a study to back me up, I believe that I noticed a decrease in amnionitis and postpartum endometritis after I left my residency. A great deal of that may be related to the fact that teaching hospitals care for sicker patients who require more intervention. In spite of that I believe some percentage of these infections come from the multiple examinations by multiple examiners that are part of care in teaching institutions. The work of Lenihan[4] concerning prelabor cervical examinations and premature rupture of the membranes had some far-reaching effects in our practices. In my office we have stopped routine weekly assessment by vaginal examination from 36 weeks because of that study. Should we then examine weekly, from much earlier on, the very patients believed to be more likely at risk of premature rupture of the membranes and amnionitis? Fifty-six percent of the study patients had preterm delivery. Does this represent an improvement over the preterm delivery rate expected, and if not, could infection play a part?

In this regard it would be good to know exactly how these examinations were done. Are the patients prepped for a sterile vaginal examination, as we were taught in our residencies, or are they examined with a sterile glove and K-Y brand lubricating jelly or perhaps Betadine povidone-iodine gel? Were all examinations conducted the same way? Was each examination an intracervical one or are paracervical examination and visual inspection sufficient and less risky?

The interest of Newman et al.[5] in premature labor, the influence of fetal number on premature labor,[6] and the management of multiple pregnancy[7] are well documented in the literature. It has been a pleasure to consider in more detail the entire topic. I want to thank the authors for their continuing efforts to quantify the risk and help us with management of this difficult obstetric problem.

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DR. JOHN W.C. JOHNSON, Jacksonville, Florida. I want to congratulate the authors on what I think is a very interesting study. This looks like a simple way to identify patients at high risk. I wondered if they have compared their approach to home monitoring, and could Dr. Newman give us some information about the relationships and interactions of these two methods of monitoring?

Dr. Leslie W. Beadling, Jacksonville, Florida (Official Guest). I was wondering if the authors have considered using ultrasonography, rather than doing vaginal examinations, to arrive at cervical scores?

Dr. Ernest H. Brown, Jr., Lumberton, North Carolina. With scores of ≤0 were cervical cultures done and were antibiotics considered in management?

Dr. Henry R. Temple, Wilmington, North Carolina. I would like Dr. Newman to comment on the five elective preterm deliveries and how these affect the overall results, if they are eliminated from the study.

Dr. Newman (Closing). The elective preterm deliveries were excluded from the analysis; therefore they have no impact on the results we presented. We did not do cervical cultures on the patients found to have advanced cervical change. This is a good suggestion and something we need to consider. One of our interests had been to perform cervical cultures to see if the antepartum cervical examination changes the bacteriologic milieu. This will be done in the future.

The use of ultrasonography for the evaluation of the cervix is intriguing. We did not have this capability in our clinic because of a lack of a vaginal probe. A word of caution is warranted in that the vaginal probe examination requires the blind introduction of a rigid and blunt instrument into the vagina. Because of manual dexterity, a digital examination actually might be a safer procedure.

We have not related our cervical change information to home uterine activity monitoring. The vast majority of the patients in this study were not performing home uterine activity monitoring. We hope to study the natural history of cervical change simultaneously with changes in uterine activity. The graph that shows the fall in cervical score with advancing gestation probably could be superimposed inversely on a graph showing increased baseline uterine activity in multiple gestations. The cervical score may prove to be useful in identifying women who are candidates for home uterine activity monitoring for the identification of increased uterine activity and more effective suppression of that activity.

With respect to the reliability of multiple examinations, I have referenced work by Stubbs et al.[1] from our own institution in which two different examiners in our antepartum clinics demonstrated good correlation in their assessment of cervical dilatation and effacement. Ninety percent of the time there was a difference of <1 cm in their measurement of cervical dilatation and in only 2% of >350 paired examinations was there a difference in dilatation of >1 cm. In only 7% of the paired examinations was there as much as 1 cm difference in cervical length. Stubbs et al. concluded that the hand is a very reliable and sensitive instrument for cervical examinations. The issue regarding tocolytic therapy for multiple gestations is significant and needs to be answered. When we do identify an abnormal cervical score, we use betamethasone prophylactically for the enhancement of fetal lung maturation. We also use the cervical score as a guideline for determining the extent of activity restriction and bed rest. A patient with a fall in cervical score from one week to the next is frequently sent to our labor and delivery unit for monitoring of possible preterm labor.

The risk of the antepartum cervical examination has been addressed in the literature by Lenihan.[2] It is important to note that his examinations were performed after 36 weeks' gestation, and I suspect they were performed in an effort to induce labor by the stripping of membranes. The preponderance of evidence suggests no adverse outcome as a result of a gentle antepartum cervical examination. I would add that the successful preterm birth prevention programs reported by Papiernik et al.[3] in France, Meis et al.[4] in North Carolina, and Herron et al.[5] in California all used antepartum cervical examinations. In their reports, albeit on singleton gestations, they showed no increase in the risk of premature delivery. In fact, they showed a reduction in the preterm delivery rates. In performing our examinations, we did not prep our patients. The examinations were performed with sterile gloves and K-Y brand lubricating jelly. At our institution we do an internal cervical examination as opposed to examining only the outside of the cervix. The examining finger is not passed beyond the point at which some resistance is identified.

Can the same information be gained by examining the external cervical os? I am not sure. Cervical effacement is evaluated by examining the outside of the cervix. However, I don't believe you can determine cervical dilatation by examining only the external portion of the cervix.

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Appropriateness of intrapartum fetal heart rate management and risk of cerebral palsy

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Cerebral palsy affects 2 in 1000 infants in the United States, and the intrapartum period is frequently scrutinized as the etiologic source. In a matched group of 49 infants with cerebral palsy at 1 year of age and 49 controls, no difference in the incidence of inappropriate intrapartum fetal heart rate pattern management was detected. This supports the conclusions of others that the intrapartum period is an infrequent source of cerebral palsy. (AM J OBSTET GYNECOL 1991;165:272-7.)

Key words: Cerebral palsy, fetal heart rate patterns, intrapartum management

Cerebral palsy is an infrequent event affecting 2 in 1000 infants in the United States.[1] Studies from the United States of births in the 1950s and 1960s[2] and from Australia of births in the 1970s and 1980s[3] investigating antecedents of cerebral palsy concluded that most cases have no identifiable antecedent events during the intrapartum period. Other studies[4, 5] have shown slight or no reduction in cerebral palsy rates after the advent of electronic fetal monitoring, which also suggests that intrapartum events are infrequent causes of cerebral palsy. In reviewing the prenatal and intrapartum courses of 34 infants in whom cerebral palsy developed, Niswander et al.[6] found no association between substandard obstetric care or delayed reaction to apparent intrapartum asphyxia and the development of cerebral palsy.

In spite of mounting evidence that cerebral palsy is rarely associated with or caused by intrapartum events, including the management of various patterns of the fetal heart rate (FHR), the intrapartum time period continues to be scrutinized as the likely source of many cases of cerebral palsy. This belief implies that appropriate intrapartum management of the FHR and its various patterns should rarely be associated with cerebral palsy.

Because of concerns regarding the quality of intrapartum care and resultant cerebral palsy, we asked the following question: Do babies in whom cerebral palsy develops have a higher incidence of inappropriate intrapartum management of nonreassuring FHR patterns than babies in whom cerebral palsy does not develop?

Material and methods

A blind, retrospective review of intrapartum management and fetal monitor strips was conducted by two perinatologists (P.J.M. and J.M.E.) for 50 infants with a diagnosis of cerebral palsy at 1 year of age and 50 matched controls. The 100 infants studied were born between 1983 and 1989 at Forsyth Memorial Hospital in Winston-Salem, North Carolina. All study infants with cerebral palsy were chosen from a group of newborns who required neonatal intensive care and who were examined at 1 year of age by one author (K.L.K.). The diagnosis of cerebral palsy was based on the finding of a motor impairment, independent of other developmental impairments. Abnormalities of position and posture were ascertained during independent evaluations by a developmental pediatrician and a pediatric physical therapist. These evaluations occurred on the same clinic date. Occasionally, serial clinic evaluations over the first year of life were required to firmly establish the diagnosis of cerebral palsy. Abnormal tone was the predominant clinical finding. Involuntary movements were not observed because of the age of the children at diagnosis. A control infant was matched for each study infant by review of obstetric delivery logs and choosing the next newborn with matching maternal race, gestational age within 1 week, and birth weight within 225 gm. Only singleton pregnancies were reviewed. The presence of specific antepartum and intrapartum factors related to cerebral palsy[2] also was evaluated. The two perinatologists, who were blind to the subject's status as a case or control, independently reviewed the intrapartum management and fetal monitor strip of each newborn and determined if specific nonreassuring FHR patterns were present and whether management was appropriate or inappropriate. If

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there was a discrepancy in their assessment of the appropriateness of care, the tracings were then reviewed by the perinatologists together, still blind as to whether the infant was a case or control newborn, to arrive at a consensus about appropriateness of care. All fetal monitor strips from each infant were reviewed, but only the 24 hours immediately preceding delivery was considered in the evaluations.

A FHR tracing was considered nonreassuring when one or more of the following was present: (1) prolonged bradycardia, defined as >2 minutes of FHR <90 beats/min; (2) recurrent late decelerations, defined as >1 hour of late decelerations in >50% of contractions in the absence of uterine hypertonicity; (3) severe variable decelerations, defined as >20 severe variable decelerations (<60 beats/min for >60 seconds' duration or >60 beats/min drop from baseline) during the course of labor.

Appropriateness of care was modified from Niswander et al.[6] No intervention was considered necessary in the intrapartum period when the following occurred: presence of reassuring FHR tracing with FHR accelerations and normal short-term beat-to-beat variability or presence of mild variable decelerations, <1 hour of late decelerations, <20 severe variable decelerations, or bradycardia to 90 beats/min for <2 minutes, as well as absence of the nonreassuring patterns previously described.

Appropriate management for nonreassuring FHR tracings was considered as follows: severe variable decelerations >1 hour or late decelerations >1 hour in >50% of contractions—maternal oxygenation and repositioning, discontinuation of oxytocin augmentation when present, fetal scalp blood pH testing if decelerations persisted in spite of interventions, and prompt delivery if the nonreassuring pattern did not subside, particularly if short-term beat-to-beat variability was absent with a fetal scalp blood pH <7.20 or if no pH was attainable and delivery was not imminent. The management of thick meconium was considered appropriate if a scalp electrode was placed, oxygen was given with a nonreassuring FHR tracing, and the above-described scheme was followed in the event of a nonreassuring tracing.

Inappropriate management was considered to have occurred when a deviation from the above recommendations occurred with a nonreassuring FHR tracing.

The electronic fetal monitor strip of one infant with cerebral palsy was misplaced during the study, and that pair was removed from final analysis. Of the 49 remaining infants in each group, five infants in the cerebral palsy group and three in the control group had a rapid admission-to-delivery interval of <60 minutes ($n = 5$) with no electronic fetal monitoring, had a scheduled repeat cesarean section ($n = 1$) with no electronic fetal monitoring, had <30 minutes of electronic fetal monitoring before emergency cesarean section ($n = 1$), or was delivered by an emergency cesarean section on hospital day 6 with no electronic fetal monitoring after two previous days of normal FHR pattern by electronic fetal monitoring ($n = 1$). In these eight infants only the presence of bradycardia and variable decelerations as documented by nursing or physician attendants was reported, because late decelerations cannot easily be detected by auscultation or a brief electronic fetal monitoring period as in these eight infants. In all 98 remaining cases nursing notes and intrapartum records were available for review.

Statistical analysis was done with Student's *t* test and χ^2 test or Fisher's exact test (one-tailed) where appropriate.

Results

Table I presents the population characteristics of the 98 infants. No statistically significant differences were noted in the mean maternal age, race, gravidity, mean gestational age at birth, or mean birth weight. Antenatal and intrapartum factors associated with cerebral palsy were found in equal frequencies in both groups. Table II gives delivery characteristics of the two groups. There was a statistically significant difference in the Apgar scores at 5 minutes ($p < 0.04$) but not at 1 minute. In 27 infants in the cerebral palsy group and 21 infants in the control group, umbilical cord blood gas values were available for review. Although 22% of the cerebral palsy group had umbilical arterial pH levels <7.20 versus 5% in the controls, the difference was not significantly different in these small groups. More newborns with cerebral palsy than control newborns also had umbilical vein pH levels <7.20 (18% vs 0%), and this difference was statistically significant ($p < 0.05$).

Nonreassuring FHR tracings occurred in 35% of the control infants and 31% of the cerebral palsy infants. Table III shows the occurrence of various nonreassuring FHR patterns and the number of patients with inappropriate intrapartum management. Similar results in Table II (delivery characteristics) and Table III (intrapartum FHR patterns and management) were seen when the eight patients with no or inadequate electronic fetal monitoring strips and their matched infants were excluded from the analysis.

Comment

Since 1862, when orthopedist William Little[7] proposed that the origins of cerebral palsy were in the circumstances of birth, many have believed that most cases of cerebral palsy arise from intrapartum events. The logical correlate to this belief is that improved intrapartum care would greatly reduce the incidence of

Table I. Population characteristics

	Cerebral palsy group (N = 49)	*Control group (N = 49)*	*p Value*
Maternal age (yr, mean)	24.6	25.1	NS
Race (No.)			
White	31	31	
Black	18	18	
Gravidity	2.2	2.5	NS
Gestational age (wk)			
Mean	29.8	29.9	NS
Range	24-41	24-41	
Preterm deliveries (≤37 wk) (No.)	44 (90%)	42 (86%)	NS
Birth weight (gm)	1513	1532	NS
Breech presentation (No.)	15 (31%)	17 (35%)	NS
Maternal mental retardation (No.)	0	0	
Placental previa	3 (6%)	2 (4%)	NS
Abruptio placentae	8 (16%)	4 (8%)	NS
Severe proteinuria*	4 (8%)	2 (4%)	NS
Intraamniotic infection	6 (12%)	4 (8%)	NS

*>5 gm/24 hours or 4+ on single voided specimen.

cerebral palsy. Unfortunately, many studies have failed to support these notions.

Nelson and Ellenberg[2] in the Collaborative Perinatal Project of the United States and Blair and Stanley[3] in Western Australia showed remarkably similar rates of cerebral palsy (<10%) when adverse intrapartum events or intrapartum asphyxia was the only obvious risk factor. In another analysis of the Collaborative Perinatal Project data, Naeye et al.[8] reported that birth asphyxia accounted for only 6% of the cerebral palsy cases in the Collaborative Perinatal Project study as a whole. Most cases of cerebral palsy in these reports appeared to originate either prenatally or postnatally.

Reviews of trends of the incidence of cerebral palsy before and after the advent of electronic fetal monitoring also have failed to demonstrate the dramatically falling rates of cerebral palsy that were originally predicted. In Western Australia rates of cerebral palsy were remarkably stable before and after electronic fetal monitoring was utilized.[9] In Sweden[5] rates of cerebral palsy actually increased slightly. Only in the United States, in a report from Wisconsin,[1] has the incidence of cerebral palsy decreased after the institution of intrapartum electonic fetal monitoring. This decrease in cerebral palsy incidence was limited to the neonate with a birth weight >2500 gm.

Other studies also suggest a lack of association between intrapartum events and the occurrence of cerebral palsy. Nelson and Ellenberg[10] reported in 1981 that 75% of term infants with cerebral palsy had 5-minute Apgar scores of ≥7. Painter et al.[11] followed the neurologic development of a group of children with abnormal FHR decelerations during labor and found all infants without genetic syndromes to be normal at age 6 to 9 years. Severe acidosis at birth in appropriate-for-gestational-age term infants in The Netherlands was not found to be a major cause or predictor of later neurologic abnormalities.[12] Even studies by Myers,[13] in monkeys in which acute hypoxia was induced, failed to consistently produce a clinical picture like cerebral palsy.

In this study the intrapartum courses and FHR patterns of 49 infants with cerebral palsy diagnosed at 1 year of age and 49 matched controls were reviewed and reported. Similar rates of nonreassuring FHR patterns as defined in this study were seen in both groups. Interestingly, more of the control infants had evidence of inappropriate FHR management during labor than did infants who later showed evidence of cerebral palsy (16% vs 10%, p not significant). This similar occurrence rate of nonreassuring intrapartum FHR patterns in both groups of infants is consistent with numerous other reports implying a prenatal (but not intrapartum) etiologic source of cerebral palsy in most cases. The only intrapartum FHR pattern in this study showing a possible trend toward association with subsequent cerebral palsy was bradycardia ($p = 0.059$), which has been reported in earlier studies.[2]

In this study significantly more 5-minute Apgar scores ≤6 were seen in the cerebral palsy group than in the control group. Also more babies with cerebral palsy had umbilical arterial or venous pH levels <7.20, although these differences were statistically significant only for the venous pH results. No difference was seen in the 1-minute Apgar scores. Because the gestational ages were matched and similar resuscitation methods were used for all babies in need, the lower 5-minute scores may reflect simply the increased incidence of acidosis in the babies that ultimately were diagnosed as having cerebral palsy. The trend toward more acidosis in babies in the cerebral palsy group may reflect our present inability to diagnose all intrapartum fetal aci-

Table II. Delivery characteristics

	Cerebral palsy group (N = 49)	*Control group (N = 49)*	*p Value*
Cesarean section rate (%)	57	49	NS
Apgar score			
1 min ≤ 2	15	9	NS
5 min ≤ 6	19/48*	9	$p < 0.04$
pH <7.20 (No.)			
Umbilical artery	6/27 (22%)	1/21 (5%)	NS
Umbilical vein	5/28 (18%)	0/22 (0%)	$p < 0.05$

*One 5-minute Apgar score was not recorded on the chart.

Table III. Intrapartum FHR pattern events and management

	Cerebral palsy group (N = 49)	*Control group (N = 49)*	*p Value*
Total nonreassuring FHR tracings (No.)	15 (31%)	17 (35%)	NS
Bradycardia >2 min (No.)	4 (8%)	0	NS (0.059)
Late decelerations >1 hr (No.)	10/44 (23%)	15/46 (33%)	NS
>20 Severe variable decelerations (No.)	2 (4%)	2 (4%)	NS
Inappropriate intrapartum management (No.)	5 (10%)	8 (16%)	NS

dosis with electronic fetal monitoring, especially if cerebral acidosis is not present. Others have shown that even severe acidosis at birth is a poor predictor of subsequent neurologic dysfunction.[14]

Although this report includes the largest series of infants studied to date for appropriateness of intrapartum FHR management and development of cerebral palsy, potential weaknesses exist.

One potential weakness is the choice of FHR patterns defined as nonreassuring, as well as the relatively short duration used in the definition. In a randomized study comparing electronic FHR monitoring to intermittent auscultation in a group of premature infants,[15] the risk of cerebral palsy was increased as the duration of the abnormal FHR pattern increased in the electronically monitored group. Had the nonreassuring FHR changes been defined differently or taken place over a longer period of time than defined in this study (for example, late decelerations for >1 hour), more evidence of fetal damage may have occurred. The time frames used were arbitrary attempts to define a reasonable period of time in which most physicians would act rather than a delimiting time in which damage does occur. A second potential weakness of the study is the relatively small number of cases and controls used. When no difference between two groups is shown, caution must be taken to prevent a type II error—not recognizing that a true difference exists between groups because of the small sample size. The prevalence of cerebral palsy is 0.2% in all births and approximately 4% in births <2500 gm.[1-9] Assuming a 4% incidence of cerebral palsy in this population with mean birth weight <2500 gm and assuming inappropriate FHR intrapartum management results in ≥50% of cases of cerebral palsy, the power of this study is >99%. However, if inappropriate intrapartum managment as defined in this study is assumed to result in only a 10% rate of cerebral palsy, the power is reduced to only 12%. To detect a difference of this magnitude with a power of 80% would require 603 pairs of infants.

This study does not imply that intrapartum FHR managment is unimportant or may not possibly lead to fetal damage in some cases. Several studies have demonstrated that, with a clearly asphyxiated newborn, damage may occur.[10, 16-18] Rather, this study suggests that most infants who survive neonatal intensive care and develop cerebral palsy have appropriately managed intrapartum FHR patterns and that inappropriate intrapartum FHR management occurs with equal frequency in infants in whom cerebral palsy develops and in normal newborns. Whereas we should continue to improve our intrapartum FHR management skills, we should remember that even the most skilled intrapartum care may not prevent the development of most cases of cerebral palsy.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ALEXANDER R. SMYTHE II, Columbia, South Carolina (Official Guest). This article touches on one of the most sensitive questions the obstetrician faces in today's medical-legal climate. Does the intrapartum period cause brain-damaged infants and cerebral palsy, or can it be absolved of blame?

This study attempts to take a small number of patients over a period of 6 years, compared with controls, and look at a nebulous question of whether inappropriate obstetric management had an influence on the incidence of cerebral palsy.

The results of this study showed no increase in cerebral palsy in patients who were deemed to be inappropriately managed in the control group and the group in whom cerebral palsy developed by definition. The incidence of cerebral palsy is small, 0.2% of all live births, and reported to be 4% in infants <2500 gm. Consequently, it would take a study of the magnitude that the authors have mentioned, somewhere on the order of 500 to 1000 patients born with cerebral palsy, to establish some cause-and-effect relationship.

The incidence of cerebral palsy has plateaued in the last 20 years with a decrease in birth trauma because of difficult operative vaginal deliveries. The mark of a good obstetrician used to be his ability to deliver an infant in the face of the most difficult vaginal circumstances. This study reminds me of the article by Schifrin and Dane[1] where they attempted to predict Apgar scores by reviewing FHR monitoring strips 30 minutes before delivery. Their study revealed two pertinent points. If the FHR monitor strip looked absolutely normal and reassuring, it had a very high index of predictability of a good Apgar score. If the FHR strip was very unreassuring and a poor Apgar score was predicted, only 20% of patients predicted to have a poor score did so, but all of the infants born with poor Apgar scores were included in this group. Consequently, the diagnosis of fetal distress was overdiagnosed in many cases in infants who were born in good health.

If we could appropriately manage even the 12% of patients in this study in whom cerebral palsy developed, we might reduce the current incidence of cerebral palsy by a fractional amount. If we assume for a moment that labor lasts 24 hours and a normal gestation is 270 days, we are looking at a small amount of time in a newborn infant's span (0.36%). This is a small blip on the continuum of life, and many studies mentioned by the authors and others have concluded that 90% of cerebral palsy is prenatal or postnatal and only 6% to 10% can be attributed to intrapartum events.

Genetic and chromosomal factors, prematurity, multiple pregnancy, hypertensive proteinuria, anomalies, bleeding disorders during pregnancy, and prenatal infections account for the majority of cases of cerebral palsy. We, as obstetricians, need to continue to improve our prevention and treatment of preterm labor, chorioamnionitis, neonatal sepsis, and seizures to reduce the incidence of cerebral palsy from an obstetric and pediatric standpoint. Prematurity with its incidence of intraventricular hemorrhage is responsible for a large percentage of patients in whose infants cerebral palsy develops.

This is reflected in the authors' study by a mean gestational age of 29.9 weeks and a mean birth weight of 1530 gm. This study also reveals the fact, once again, that cesarean section does not protect against the development of cerebral palsy and should not be used in preterm deliveries unless obstetric guidelines dictate.

An excellent study by Grant et al.[2] presented at the World Perinatal Symposium and published in *The Lancet* in 1989 involved a randomized trial of 13,079 liveborn children. Electronic fetal monitoring was compared to intermittent auscultation to determine if fetal monitoring had any protective effect against cerebral palsy.

There was no difference between the groups in the overall death rate, but neonatal seizures were found significantly less often in the monitored group. Again, this study indicated that suboptimal intrapartum care (in particular, failure to respond appropriately during labor to an abnormal FHR pattern) was shown to be associated with about a threefold increase in the risk of very early neonatal seizures but not with an increased risk of cerebral palsy.

Again, I would like to commend the authors on an excellent study showing that even inappropriate responsiveness to FHR abnormalities as defined by them failed to cause an increase in the incidence of cerebral palsy. This is an important topic and will continue to bewilder the legal and medical field until we can understand and study in utero the developing fetus to a much closer degree.

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Dr. Charles J. Ward, Decatur, Georgia. I commend the physicians on this study. It will be very helpful not only for our profession but for those who enter the legal arena to defend physicians in cases of cerebral palsy. I would ask if the placenta was retained in any of the cases? We have learned that an abnormal proliferation of trophoblasts in the spiral arteries at about 16 to 18 weeks of gestation reduces the flow to the intervillous space and is associated with villous edema. Were the placentas saved in any of these cases and, if so, are they still available for study?

Dr. Mason C. Andrews, Norfolk, Virginia. Dr. Smythe pointed out the importance of prematurity in this condition. The study is reassuring about our obstetric care. I wonder if the authors could tell us how many of the 50 cases had intraventricular hemorrhage?

Dr. Ernest (Closing). I think it is appropriate to remember that labor involves only 0.36% of an entire gestation. People wonder whether cerebral palsy is related to labor or to the process of prelabor, which is much longer. It is reassuring that Grant et al. reported that neonatal seizures, while frightening, usually do not correlate with subsequent neurologic damage.

The placentas were not retained. As things evolve and as we learn more, I believe that when we deliver a baby that "looks funny," has meconium in the amniotic fluid, or is preterm, we ought to at least evaluate umbilical cord blood gas values and probably send the placenta for pathologic examination so that we can save it and look at it later if the case "comes back to haunt us" in court.

I do not know the number of babies who had intraventricular hemorrhage, but I think it is also an important consideration.

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Resolution of chronic pelvic pain after laparoscopic lysis of adhesions

John F. Steege, MD, and Anna L. Stout, PhD
Durham, North Carolina

Thirty women undergoing laparoscopic lysis of adhesions for the treatment of chronic pelvic pain were prospectively evaluated for the presence of a chronic pain syndrome. At follow-up, of those with chronic pain syndrome (10), four (40%) reported continued improvement or resolution of pain during daily activities or dyspareunia, whereas of those without chronic pain syndrome (20), 15 (75%) were better ($p = 0.06$). When these two complaints are evaluated individually, both pain during daily activities ($p < 0.05$) and dyspareunia ($p < 0.05$) are more likely to improve after lysis of adhesions in women without chronic pain syndrome. Prognosis was not related to the number of previous operations, adhesion score, or other physical parameters. Laparoscopic lysis of adhesions is generally worthwhile in the treatment of chronic pelvic pain, although the presence of psychosocial compromise warrants preoperative evaluation and concomitant treatment. (AM J OBSTET GYNECOL 1991;165:278-83.)

Key words: Chronic pelvic pain, adhesions, operative laparoscopy

Of all operative laparoscopy procedures performed in the United States, about 40% are done for the evaluation and treatment of pelvic pain.[1] In approximately 30% to 50% of chronic pain cases, pelvic adhesions are found,[2-4] although their relationship to pain is uncertain.[5, 6] We recently reported that the location of pain correlates well with the location of the pathologic condition (usually adhesions or endometriosis),[7] although the available clinical studies do not demonstrate a quantitative relationship between adhesions and chronic pelvic pain.[7, 8]

Studies of other chronic pain problems such as low back pain have similarly failed in the attempt to quantitatively associate pain with a pathologic condition.[9] Together with other observations,[10] these studies point out the inadequacies of the Cartesian theory of pain perception, which hypothesizes unique pain fibers and holds that pain is proportional to tissue damage. Newer theories of pain perception such as the gate control theory and cognitive models include the influences of culture, psychologic conditioning, cognitions, affect, and stress.[11] Considering these complexities, it should perhaps not be surprising that attempts to find enough pathologic conditions to explain chronic clinical pain have routinely been frustrating. Clearly, some patients get negative or nociceptive input of substantial degree from their illnesses but never develop the affective, behavioral, and cognitive hallmarks of what we have called a chronic pain syndrome.[12] When these behavioral and psychologic changes do occur, to what degree is it useful to treat conditions, such as pelvic adhesions, that rarely affect general physical health?

This study of laparoscopic lysis of adhesions assesses outcome in terms of relief of daily pain and dyspareunia and examines the relationship between pain relief and the preoperative diagnosis of a chronic pain syndrome.

Methods

Thirty-four women aged 18 to 70 years (mean, 34.9 ± 9.9 years) were evaluated in a teritiary care medical center for chronic, constant pelvic pain of ≥6 months' duration. Consecutive patients who ultimately proved to have pelvic adhesions at laparoscopy were included in the present study. All women submitted documentation of previous medical care, including surgical procedures, and completed detailed clinical interviews and physical examinations. Of the 30 women reached for follow-up evaluation, one had previously had unilateral salpingo-oophorectomy, whereas 19 had undergone hysterectomy, 10 with bilateral and five with unilateral salpingo-oophorectomy.

At their first visit patients rated dysmenorrhea, dyspareunia, and pain during daily activities on a numerical scale of 0 to 10, with the anchors of 0 being "no pain" and 1 being the "worst pain I can imagine." The following parameters of the pain were then scored by the clinician as present or absent: (1) pain duration of ≥6 months, (2) incomplete relief by previous treatments (e.g., analgesics, prior operations), (3) impaired

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6/6/30392

Table I. Adhesion scores and pain ratings in patients with and without chronic pain syndrome

	With chronic pain syndrome	*Without chronic pain syndrome*	*Total*
No.	10	20	30
Adhesion score	6.3 ± 4.2	7.5 ± 3.3	7.0 ± 3.57
Pain during daily activities			
Preoperative rating	8.56 ± 1.42 (n = 9)	7.63 ± 1.83 (n = 16)	7.96 ± 1.96 (N = 25)
Postoperative rating	6.56 ± 2.79*	2.44 ± 2.97*	3.92 ± 3.56
Postoperative course†			
Improved	3/9 (33%)	11/16 (69%)	14/25 (56%)
Unchanged	3/9 (33%)	1/16 (6%)	3/25 (12%)
Improved, then pain returned	3/9 (33%)	4/16 (25%)	8/25 (32%)
Dyspareunia			
Preoperative rating	7.67 ± 1.97 (n = 6)	8.5 ± 1.6 (n = 14)	8.25 ± 1.71 (N = 20)
Postoperative rating	6.4 ± 2.70*	2.5 ± 2.53*	3.52 ± 3.06
Postoperative course†			
Improved	2/6 (33%)	5/14 (36%)	7/20 (35%)
Cured	0	7/14 (50%)	7/20 (35%)
Unchanged	3/6 (50%)	2/14 (14%)	5/20 (25%)
Worse	1/6 (17%)		1/20 (5%)
Daily pain and/or dyspareunia‡			
Improved	4	15	19
Not improved	6	5	11

*$p < 0.05$.
†$p < 0.05$, combining all nonimproved categories.
‡$p = 0.06$.

physical function (work, recreation, etc.), (4) at least one vegetative sign of depression (sleep disturbance aside from being awakened by pain, loss of appetite, or psychomotor retardation), and (5) altered family roles. The last category included child care and household responsibilities, financial decision making, and emotional involvement with and support of other family members. Women were designated as having a chronic pain syndrome if four or more of these characteristics were present.

All the women underwent operative video laparoscopy while they were under general anesthesia, using a carbon dioxide laser, scissors, hydrodissection, and blunt dissection to lyse adhesions. At the termination of each procedure, Interceed (Johnson & Johnson, Newark, N.J.) adhesion barrier was placed over areas of dissection along the pelvic sidewalls and moistened with 4 to 6 ml of heparin, 1000 U/ml. Patients returned for follow-up examinations at 1 month and again at intervals ranging from 6 to 12 months (mean, 8.2). At follow-up all patients either scored pain during a structured clinical interview or completed a questionnaire including the same rating scale items.

Before tabulation of the clinical data on pain severity and chronic pain syndrome classification, all videotapes of the laparoscopic procedures were reviewed and adhesions were scored in the following areas: (1) right ovary, (2) right tube, (3) left ovary, (4) left tube, (5) omentum, (6) large bowel, (7) small bowel, (8) pelvic sidewalls, and (9) cul-de-sac. The severity of adhesions was rated as follows: 0, none; 1, filmy; 2, dense or vascular; and 3, dense and vascular. Dense was defined as opaque, and vascular as bleeding on laser dissection or, in the judgment of the operator, necessitating electrocoagulation before severing with scissors or laser. A total adhesion score was then calculated by summing scores across all nine areas evaluated. Adhesion location was compared with pain location, with reported pain characterized as right, left, central, or any combination of these areas.

Patients were then categorized by the presence or absence of chronic pain syndrome, and the resulting two groups were compared across demographic variables, adhesion scores, and clinical outcome at 6 to 12 months (mean, 8.2) after surgery. Categoric variables were compared with the χ^2 test or Fisher's exact statistic and continuous variables with the Student t test and Pearson's r; $p < 0.05$ was used as the level of significance.

Results

Ten patients had a chronic pain syndrome and 20 patients did not. By virtue of the inclusion criteria for the study, all patients fulfilled the first two criteria for a chronic pain syndrome (duration of ≥6 months, incomplete relief by previous treatments). In addition, 19 had impaired physical function, nine had at least one vegetative sign of depression, and seven acknowledged significant changes in their roles within the family.

Of the 30 patients completing evaluation, 25 had had laparotomy (mean ± SD, 1.55 ± 1.27; range, 1 to 4),

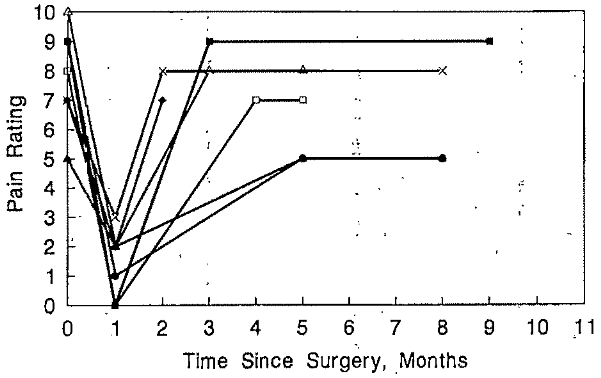

Fig. 1. Time of return of pain during daily activities after laparoscopic lysis of adhesions (n = 8).

and 10 had had laparoscopy (mean ± SD, 0.52 ± 0.83; range, 1 to 3). Patients with chronic pain syndrome more often had prior operations (laparotomy, 1.9 vs 1.36; laparoscopy, 0.8 vs 0.36) and hysterectomy (80% vs 55%), but neither of these differences reached statistical significance in this sample. Patients with and without chronic pain syndrome had similar preoperative ratings of dyspareunia and pain during daily activities, as well as similar adhesion scores (Table I). Because of the paucity of women with chronic pain syndrome and intact uteri (n = 2), the effects of laparoscopic lysis of adhesions on dysmenorrhea could not be compared between the two groups.

Pain location overlapped with the location of adhesions in 90% of patients; both were scored by dividing the pelvis in thirds (right, left, and middle). Preoperative ratings of dyspareunia and pain during daily activities did not correlate with adhesion scores in the study as a whole or in the patients with or without chronic pain syndrome. Small- or large-bowel adhesions were equally prevalent in the group with (80%) and without (70%) chronic pain syndrome.

Five patients had dyspareunia only, whereas 25 had pain during daily activities; 15 of these also had dyspareunia (Table I). Of the 25 patients with chronic pain during daily activities, 14 (56%) had either complete resolution of pain (n = 9, 36%) or at least 50% reduction of pain (n = 5, 20%) at follow-up. Seven (28%) had initial improvement, with pain returning to >50% of preoperative levels within 2 to 5 months of the operation (Fig. 1), and four (16%) had no improvement (Table I). The return of pain after transient relief was associated with bowel adhesions at the trend level (p = 0.19). Fourteen of 20 patients (70.0%) with deep dyspareunia had either complete resolution (n = 7, 50.0%) or improved >50% (n = 7, 50.0%) after operative laparoscopy (Table I). In some cases resolution of dyspareunia was apparently hindered by concomitant vaginal factors of tightness and deficient lubrication. In women with both dyspareunia and pain during daily activities, the postoperative courses of both symptoms were similar, i.e., either both improved or both persistent in all cases.

Patients without chronic pain syndrome were significantly more likely to have prolonged relief of pain during daily activities ($p < 0.05$) and relief of dyspareunia ($p < 0.05$) (Table I). When results with daily pain and dyspareunia are combined, these patients were more likely (at the strong trend level, p = 0.06) than women with chronic pain syndrome to obtain good results. Relief of dyspareunia and pain during daily activities was not related to adhesion score in the whole group or in the subgroups with or without chronic pain syndrome.

Comment

Models of pain perception have grown more complicated with time, in part as a response to the need for a better model to explain the complexities of chronic pain syndromes. The ancient Cartesian theory, which postulated the existence of neurons having the single function of carrying pain signals to the brain, has given way to the gate control theory[11] and to cognitive-behavioral models.[13] These models integrate the influences of personality, affect, organic changes, sensory thresholds, and cognitions about pain and its causes in a manner that avoids clear demarcation between psychologic and physical causes for pain but rather recognizes their intricate and interdependent relationships. This study suggests that lysis of pelvic adhesions may indeed be a useful procedure in many circumstances. Pain associated with adhesions is less often relieved when accompanied by a chronic pain syndrome, i.e., diminished physical activity, vegetative signs of depression (usually sleep disturbance), and substantial alteration of the patient's role in the family. The results are most consistent with a model of pain perception that integrates physical and psychologic factors.

Our study replicates our previous finding[7] that the anatomic site of the pathologic condition often matches well with the reported location of pain but that the intensity of pain does not correlate with the amount of disease present. Similar findings have been noted in the case of endometriosis.[14]

In short-term follow-up the majority (88% in this study) will report relief of pain. About one fourth will note return of pain within 3 to 5 months, emphasizing the need for long-term monitoring. Women in this study with chronic pain syndrome, more often than those without, tended (at trend level) to have pain return after transient relief. The clinical dilemma after pain returns becomes whether to pursue further lysis of adhesions or to follow other therapeutic avenues. These results support the approach that any evidence for elements of a chronic pain syndrome should prompt application of nonsurgical therapies, regardless of whether further lysis of adhesions is performed.

Several investigators have pointed out that pelvic abnormalities in general[5, 15] and adhesions in particular[6] may be no more prevalent in women with pain than in those who are pain free. The degree and location of adhesions in these comparative studies are not specified. The comparison populations in this latter study[6] were possibly self-selected as pain free, i.e., sufficiently comfortable to allow pursuit of infertility treatment, and hence were not a valid comparison sample. These studies imply that, like endometriosis, pelvic adhesions apparently do not always cause pain but are a source of nociceptive signals in many cases. This study suggests that adhesions involving large or small bowel may play a more significant role in pelvic pain than adhesions in other areas, especially in terms of the chance of recurrence of pain after laparoscopic lysis of adhesions.

Many studies have evaluated the impact of lysis of adhesions on fertility, but only a few[16-18] have examined the role of this procedure for pain relief. In primary care settings, the proportion of patients helped by lysis of adhesions may be as high as 84%,[17] and, even in the case of severe adhesions with many preceding operations, the majority may be helped.[16]

The precise role of psychosocial factors in chronic pelvic pain is uncertain. In the apparent effort to avoid confounding variables, many studies examining the psychologic profile of the pelvic pain patient have focused on "laparoscopy negative" women or compared the psychologic profiles of women with and without organic findings. Such studies have emphasized the role of psychosocial factors such as a distressed or multiproblem family of origin, sexual abuse, marital discord, and psychiatric diagnoses such as depression, anxiety, and personality disorders.[15, 19] These factors no doubt play substantial roles in many patients. We postulate that a person with such difficulties may be ill equipped to cope with the uncomfortable somatic sensations accompanying any disease process and perhaps especially vulnerable to impairment when the disease involves a body area (the reproductive or sexual system) that may have been the target of abuse. However, the observation that these factors may complicate the presentation of symptoms in the presence of organic disease does not demand the conclusion that the organic disease is of no significance. These data support the notion that lysis of adhesions is useful but should be accompanied by additional psychologic evaluation and treatment in these women displaying vegetative signs of depression or significant alteration of family roles. Further clinical studies are needed to clarify subtypes of patients with chronic pelvic pain, to understand the role for lysis of adhesions in women with significant psychosocial risk factors, and to better understand the types of nonsurgical therapies most likely to be effective.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ROBERT B. ALBEE, JR., Atlanta, Georgia (Official Guest). Pelvic pain that has no explanation is one of the most frustrating problems that the gynecologist faces. However, even more exasperating is the failure of pelvic pain to respond to the appropriate and thorough treatment of pelvic pathologic conditions.

Drs. Steege and Stout have presented a prospective comparison of consecutive patients undergoing laparoscopy for pelvic adhesions. Three preoperatively evaluated parameters are then used to isolate a subgroup of patients whose successful resolution of pain is different and less than the remainder.

In the study group all patients had pain for ≥6 months and none had complete relief of pain from previous medical or surgical treatment. The preoperatively evaluated parameters were as follows: (1) the presence of impaired physical function (work, recreation, etc.), (2) the presence of vegetative signs of depression (sleep disturbance aside from being awakened by pain, loss of appetite, psychomotor retardation), and (3) the presence of alteration of family roles.

The findings suggest that in patients with prolonged pain and previous treatment failure, there may still be good reason to try to eradicate residual adhesions; however, if two of the three above-listed conditions are present (i.e., chronic pain syndrome), the chances of long-term relief from pain are less than if those conditions are not present.

This information is going to come as no surprise to clinicians, yet there is almost nothing in the literature that prospectively evaluates these specific variables.

The authors concluded that psychologic treatment should be used in addition to the surgical lysis of adhesions if patients have diminished physical activity and vegetative signs of depression or significantly altered family roles.

I believe it also would be appropriate to counsel these individuals before operation regarding their reduced relative success compared with the group without chronic pain syndrome. This would give each individual the opportunity to reconsider any other factors that may be related to their pain. It also more appropriately defines the chances of long-term success.

Drs. Steege and Stout do not specify the types of psychologic treatment being used or recommended in these patients. Typically behavioral modification, counseling, group therapy, and drug therapy (such as anxiolytics and antidepressants) all have been used. In virtually all of these treatment avenues, definitive treatment depends on the individual's ability to acknowledge and assess all factors contributing to the pain. Thus, as the authors have recommended, we must not pass up this opportunity to encourage each patient to seek further evaluation and treatment of the other factors involved.

I would like to ask the authors if it is safe to assume that they have excluded from this study group all patients who were found to have any concomitant pelvic disease in addition to adhesions? I think it is important to point out that the validity of their conclusioins would certainly be affected by the presence of endometriosis, myomas, ovarian cysts, etc., because the prevalence of other diseases was not indicated in the two study groups.

If other diseases were excluded, I would like to ask to what extent an effort was made to rule out the presence of what is being referred to as microscopic endometriosis?[1, 2]

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DR. DOROTHY E. MITCHELL-LEEF, Atlanta, Georgia. You discussed the fact that you have encountered adhesions. What are you using for the prevention of further adhesions, and are you considering repeat laparoscopy in those patients who experience a return of pain?

DR. J. GREG JOHNSON, Greenville, South Carolina. What is the role of uterosacral ablation in the control of pain in a patient with an intact uterus?

DR. WALLACE C. NUNLEY, JR., Charlotte, North Carolina. In the six of 10 patients with chronic pelvic pain syndrome who did not respond over the long term, did you offer referrals or psychologic evaluation, and if so, would you share information as to their subsequent course?

DR. JAROSLAV F. HULKA, Chapel Hill, North Carolina. Dr. Steege is to be congratulated for pointing out the transient nature of some of the cures that we have relied on for the management of pain. His illustration showing a 1-month cure and a six-month recurrence is important in our reevaluating the literature about the efficacy of pain medication and surgery. I want to ask a question about the efficacy of psychiatric therapy. Given that the patients do not respond to surgery, what is your experience about the efficacy of psychotherapy; in other words, how can we help these patients?

DR. STEEGE (Closing). As I mentioned, we did use the Interceed adhesion barrier as a part of our standard procedure. I don't mean to say that I am convinced that it is the be-all and end-all of adhesion prevention. I think that it should be evaluated and used laparoscopically, as opposed to generalizing from its use during laparotomy, because laparotomy in itself predisposes to adhesion recurrence far more than does laparoscopy. In laparoscpic reports available so far, there is a reduction of adhesion formation by 50% to 60%, regardless of what kind of operative technique is used. Whether you use Hyskon or Interceed, the results are similar.

The use of uterosacral ablation applies primarily to dysmenorrhea. I don't think its use applies to chronic pelvic pain. Nineteen of 30 patients in our series had hysterectomy. Of those who had pain, seldom was it totally central pain, and that is because these are patients with adhesions only. Although there were some with adhesions to the fundus of the uterus, most of them had adhesions involving the adnexa and bowel. Seventy-five percent of the total group had adhesions involving the large bowel, small bowel, or both. I believe that uterosacral ablation is useful only for central pelvic pain. The nerve supply of the adnexa and the pelvic sidewalls doesn't go through the uterosacral ligaments.

The follow-up of nonresponders is difficult because they are relatively few and because they are a very heterogeneous group in terms of their social situations and their psychiatric histories. I should mention that one obvious thing missing in this article is any assessment of whether sexual abuse, during either childhood or adult life, had been a part of their history. I think that many of you are aware of recent studies showing that in women experiencing chronic pelvic pain, defined in a very similar way—≥6 months—about 60% had been sexually abused at some point in life. The question then becomes, "Is there a predisposing factor that makes a person vulnerable to whatever nociceptive signals come from whatever pelvic abnormality there is, or is there a current, important active ingredient that needs to be addressed?" I think that question is far from settled. Clinically, when I get that history, I have a hard time knowing quite what to do with it, on the basis of not knowing how it connects to the symptoms and having seen discouraging results in the psychotherapeutic treatment of those who have been victims of sexual abuse. Nonresponder follow-up is a far more difficult question to answer and involves much more complex levels of assessment. I think our results with those have been very mixed. We do use antidepressants, psychotherapy, inpatient psychiatric hospitalization, and a variety of different treatments. Again, it is very difficult to generate homogeneous groups to evaluate results.

There are several articles in the literature that talk about psychotherapy or behavioral therapy for the treatment of chronic pelvic pain. One was done at a military facility in which a chronic pain clinic was the ultimate referral for anyone with chronic pain. They demonstrated that the frequency of hysterectomy could be drastically reduced by such referral. The problem in applying this to general practice is that people in the military system are a captive audience. They do not have the option for private medical care, short of paying for it themselves and going outside the system to have a hysterectomy. I think that in many of our populations if we say, "You don't really need a hysterectomy," many will simply walk out and find someone else who will perform a hysterectomy. Therefore I am not sure the results of the military study apply.

Individual psychotherapy, in the sense of a typical psychodynamically oriented approach, is less likely to be useful. What is more likely to be useful, generalizing from work with chronic pain in other areas such as back pain, headache pain, etc., is a more behavior-oriented program that takes a more rehabilitative approach, maximizing what positive elements in the person's life are left, approaching the roles within the family that may have changed, looking at the role of the husband and the children, etc., in the perpetuation of the chronic pain syndrome. Thus the burden of responsibility is basically taken away from the symptom bearer and the family system is examined. I think that is more likely than individual psychodynamically oriented psychotherapy to be effective.

Combined interferon alfa and doxorubicin in the treatment of advanced cervical cancer

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Interferon alfa and doxorubicin have been shown to have synergistic effects when tested in vitro with cells derived from cervical cancers. A clinical trial was designed, testing interferon alfa plus doxorubicin in patients with advanced or recurrent cervical cancers. Twenty-one patients were given interferon alfa, 10 million units per square meter intramuscularly and 10 million units per square meter intravenously over a 30-minute infusion period. One hour later 20 mg/m^2 doxorubicin was given intravenously slowly over a 2-hour period. Treatments were repeated once weekly for 3 weeks. Initial response evaluation was done at week 5, and treatment was continued on an every-other-week schedule for patients showing favorable responses or stable disease. Bone marrow, hepatic, and renal toxicities were minimal. Fever and malaise were the major sources of toxicity. Of the 17 evaluable patients, six had clinical partial responses. Two of the responders have enjoyed more than 5 years' survival. This regimen is well tolerated and does have efficacy in some patients with advanced cervical cancers. (AM J OBSTET GYNECOL 1991;165:284-91.)

Key words: Cervical cancer, chemotherapy, interferon, interferon and doxorubicin

Advanced malignancies after failure of primary therapy are generally re-treated with only palliative goals in mind. Cancers arising in the uterine cervix that are first seen as advanced or metastatic disease are commonly treated with primary radiation therapy. Cure rates achievable with radiation therapy are reportedly as high as 65% for stage IIB disease and up to 35% for stage IIIB disease.[1] However, even under the best of circumstances, there will be many patients with persistent disease after therapy, either because of failure of radiation to sterilize bulky pelvic disease or from metastatic tumor appearing in distant sites.

Chemotherapy for primary malignancies of the cervix has had very limited success in the past. Reasons for chemotherapy failure include probable inherent resistance of tumor cells to many chemotherapeutic agents in addition to the difficulty in delivering therapeutic concentrations of drugs to an irradiated tumor bed. These realities notwithstanding, attempts continue to identify helpful treatment for patients who experience recurrent cervical cancer.

Preclinical data have demonstrated that tumor cells from patients with cervical cancers do show a limited degree of sensitivity to the antiproliferative effects of interferon alfa.[2] Further enhancement of in vitro antiproliferative effects has been shown when interferon alfa is combined with doxorubicin.[3, 4]

This report is of a phase II clinical trial that was based on the preclinical clues that combined interferon alfa and doxorubicin have antiproliferative activity in the treatment of primary cancers of the cervix. The study design, including the novel doses and schedules of drugs administered, was based on in vitro data.[5]

Material and methods

Twenty-one patients with primary cancer of the uterine cervix were treated at Bowman Gray School of Medicine of Wake Forest University, according to this protocol, using combined interferon alfa and doxorubicin. Each patient signed an informed consent approved by the Bowman Gray Clinical Research Practices Committee. Entry criteria included histologic confirmation of advanced or recurrent cervical cancer. All patients were required to have either measurable or evaluable tumor that could be assessed for response. Performance status for each patient was 0, 1, or 2 (Eastern Cooperative Oncology Group criteria[6]). Patients must have recovered from any toxicity resulting from prior radiation therapy or chemotherapy. Of these 21 patients, 14 had squamous cell type, five had adenosquamous tumor, and two had primary adenocarcinoma of the cervix. There were no patients entered with the small cell variant of cervical cancer.

The treatment schema was based on clues derived from in vitro testing of tumor cells exposed to sequen-

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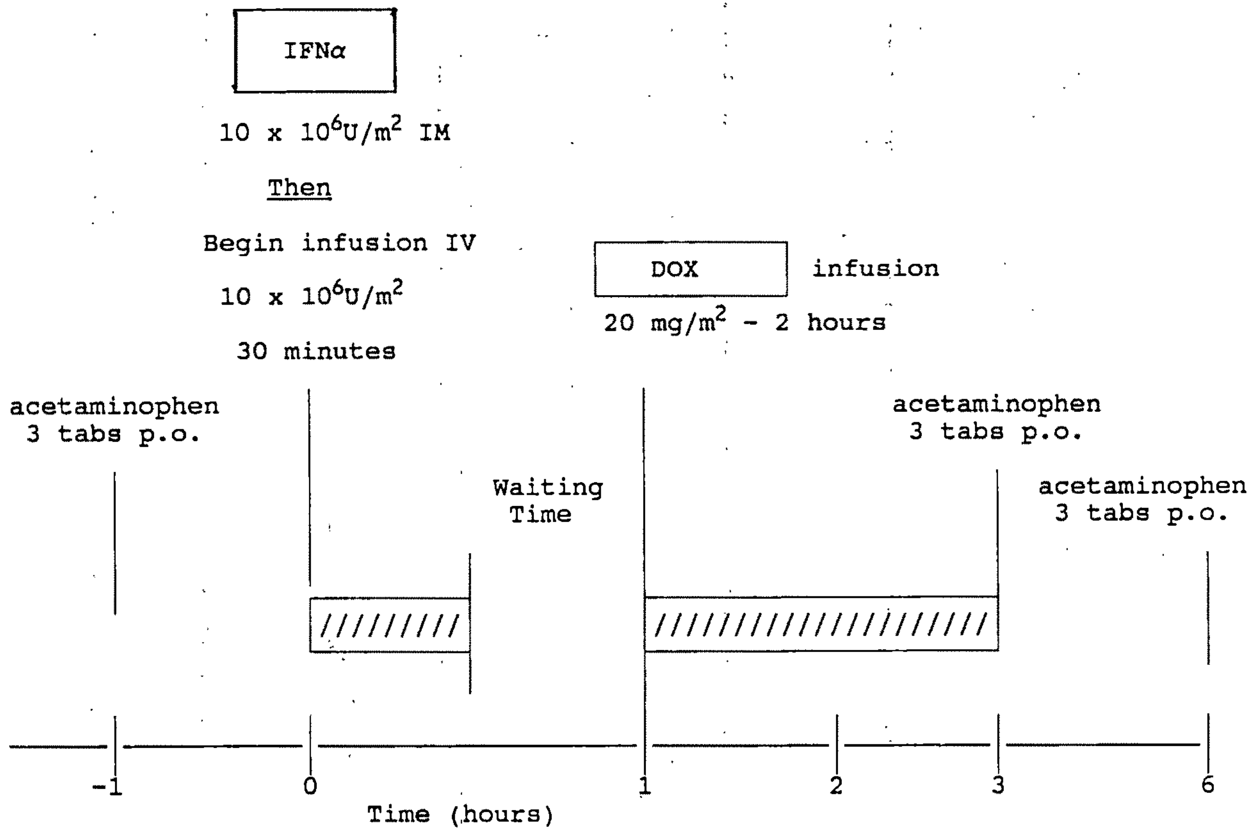

Fig. 1. Protocol schema for sequential administration of interferon alfa and doxorubicin. *IFNα,* Interferon alfa; *DOX,* doxorubicin.

tial interferon alfa and doxorubicin.[7] The purpose of the study design was to give both drugs in close sequence. Patients received treatment only when both agents could be given safely. The schedule of drug administration is summarized in Fig. 1. Interferon alfa was administered partly as an intravenous infusion of 10 million units per square meter over a 30-minute period to achieve rapid peak blood levels. An additional intramuscular injection of 10 million units per square meter also was given at the same time and served to extend the serum levels of interferon alfa for a period >8 hours. After a 30-minute waiting time, the 2-hour doxorubicin infusion was begun. The 20 mg/m² dose of doxorubicin selected was as high as was believed to be manageable on a weekly basis. The infusion was extended over a 2-hour period to prolong the levels of doxorubicin beyond those seen when rapid intravenous bolus infusion is given. Acetaminophen was given according to the schedule shown in Fig. 1. The initial treatment, referred to as the induction phase, included three weekly treatments of interferon alfa followed by doxorubicin. A 2-week rest followed, after which evaluation of initial response was made. Patients who showed any favorable response or even stable disease were continued on maintenance therapy, consisting of the same dose schedule given every second week. Patients had treatment discontinued when the disease progressed, when toxicity was noted, or when they desired to stop therapy.

Interferon alfa used in this study was provided by the Schering Corporation, Kenilworth, N.J. This interferon (Intron A) is of recombinant type and highly purified.

Background information for each new patient is noted in Table I.

For a patient to be considered evaluable in this study, she had to complete the three induction courses of treatment and have the measurable/evaluable indicator lesion reassessed for response. Response evaluation was determined by conventional definition. A complete response was determined clinically, requiring that all of the indicator lesion disappear, determined by two observations not <4 weeks apart. Partial responses were defined as >50% shrinkage of the indicator lesion, lasting at least 4 weeks. Toxicity was graded according to World Health Organization criteria,[8] particularly noting hematologic, hepatic, and renal toxicity, as well as febrile morbidity and consitutional side effects.

Results

Of the 21 patients entered onto this study, 17 were evaluable for responses. Reasons for each of the four patients declared unevaluable are summarized in Table II. Clinically determined responses to therapy were ob-

Table I. Background information of patients entered in study

Patient No.	*Age (yr)*	*Performance status*	*Cell type*	*Initial stage*	*Prior treatment*	*Comments*
1	64	2	Squamous	IIIB	Pelvic radiation therapy	Severe radiation proctitis
2	42	0	Squamous	IIB	Total abdominal hysterectomy Postop radiation therapy, Cisplatin, 5 courses	Tumor cut across
3	70	1	Squamous	IVB	No prior therapy	
4	42	2	Squamous	IIIB	Pelvic radiation therapy, bleomycin, cisplatin, mitomycin C	Pelvic recurrence
5	66	1	Squamous	IIIB	Laparotomy, bleomycin, mitomycin C	Omentum positive, paraaortic nodes positive
6	31	0	Squamous	IB	Radical hysterectomy, postop radiation therapy	Paraaortic nodes positive
7	42	1	Adenosquamous	IIB	Pelvic radiation therapy	
8	36	1	Adenosquamous	IIB	Pelvic radiation therapy	
9	42	1	Squamous	IB	Radical hysterectomy, postop radiation therapy	Nodes negative, radiation therapy pelvic recurrence
10	57	2	Squamous	IIB	Pelvic radiation therapy	
11	28	1	Squamous	IB	Pelvic radiation therapy, mitomycin C	Recurrent tumor in pelvis
12	47	1	Squamous	IB	Radical hysterectomy, cisplatin	Nodes negative, recurrence in pelvis
13	69	1	Adenocarcinoma	IB	Radical hysterectomy, postop radiation therapy	
14	63	1	Squamous	IIIB	Pelvic radiation therapy	
15	38	2	Squamous	IIB	Pelvic radiation therapy, carboplatin	Recurrent pelvic tumor
16	34	0	Adenocarcinoma	IB	Radical hysterectomy, postop radiation therapy	Nodes positive
17	56	1	Squamous	IIB	Pelvic radiation therapy	No recurrence in pelvis
18	29	1	Adenosquamous	IB	Laparotomy; postop radiation therapy; cisplatin, 7 courses; mitomycin C, 6 courses	Recurrence in pelvis and supraclavicular nodes
19	58	2	Adenosquamous	IIA	Pelvic radiation therapy	Necrosis, upper vagina
20	46	1	Adenosquamous	IIA	Radical hysterectomy, postop radiation therapy	
21	60	1	Squamous	IB	Trachelectomy, postop radiation therapy, cisplatin	Pelvic recurrence

Table II. Summary of responses of evaluable and unevaluable patients

Patient No.	*Date of original diagnosis*	*Date of entry in study*	*Site of measurable/evaluable disease*	*No. of courses of therapy*	*Best response*	*Status*
Evaluable patients						
1	11/83	2/84	Pelvic mass, bone lesion	6	Partial response	Alive 10/90
2	11/83	11/84	Vaginal mass 8 × 8 cm	7	Partial response	Alive 7/89
3	2/84	2/84	Vaginal and vulvar metastases	8	Partial response	Died 11/84
4	2/82	9/83	Pelvic mass 10 × 5 cm	12	Partial response	Died 6/84
5	5/83	11/83	Vaginal nodule 1 × 3 cm	8	Partial response	Died 4/84
6	7/83	5/85	Lung mass 1.2 × 1.5 cm	13	Partial response	Died 3/87
7	4/83	1/84	Vaginal mass 4 × 5 cm	6	Stable	Died 1/85
8	3/83	1/84	Pelvic mass, frozen pelvis	3	Stable	Died 4/84
9	5/83	11/83	Vaginal mass 1 × 3 cm	4	Stable	Died 2/84
10	8/82	10/83	Pelvic mass 8 × 10 cm	4	Stable	Died 3/84
11	4/83	11/83	Inguinal mass 2 × 4 cm	4	Stable	Died 1/84
12	2/81	10/83	Pelvic mass 4 × 6 cm	7	Stable	Died 7/85
13	10/78	1/84	Lung mass 10 × 12 cm	11	Stable	Died 9/84
14	9/82	2/84	Lung mass 2 × 2.5 cm	13	Stable	Died 11/85
15	9/83	3/84	Pelvic mass, frozen pelvis	3	Stable	Died 8/85
16	3/84	1/85	Vaginal mass 4 × 3 cm	5	Stable	Died 3/86
17	7/84	7/85	Lung mass 4 × 4 cm	10	Stable	Died 1/86
Unevaluable patients						
18	9/82	6/85	Pelvic mass 10 × 15 cm, supraclavicular node	2	Did not complete therapy	Died 7/85
19	9/83	4/85	Pelvic mass, lung nodule	2	Did not complete therapy	Died 5/85
20	3/84	12/84	Bone lesion	3	Evaluation not done	Died 1/85
21	2/81	11/84	Vaginal mass 6 × 5 cm	1	Refused therapy	Died 11/85

served in six of the 17 evaluable patients. Each experienced a partial response, noted at the completion of therapy. There are two patients of particular interest who have experienced long-term remissions. Patient 1 (see Table I) had persistent pelvic disease after primary radiation therapy for a stage IIIB lesion. She received three induction courses in February 1984, followed by three additional courses of maintenance therapy. Treatment had to be discontinued in early Mary 1984 when she had a hip fracture. On clinical evaluation she had no apparent tumor remaining but did have induration in the pelvis after radiation therapy. It was not possible on clinical examination to be sure that she was totally free of tumor. Hence she has been considered to be a partial responder. However, she remains free of any recurrence or symptoms of new disease as of October 1990. She has had no additional therapy since May 1984.

Patient 2 had stage IIB cancer discovered at the time of surgery for uterine fibroid tumors. The carcinoma was surgically cut across at the parametrial edges of the specimen and was subsequently treated with postoperative pelvic radiation therapy. The vaginal apex failed to heal after surgery and radiation, partly related to persistent parametrial tumor and the radiation effect on the tissues. She was given five courses of intravenous cisplatin chemotherapy, without any apparent antitumor response. At the time of entry into this study in November 1984, she had an 8 × 8 cm mass at the vaginal apex. After completing the 3-week induction therapy, she already had a partial response noted. She had an additional four courses of maintenance therapy. Treatment was stopped at this point when surgery was required because of a colon obstruction. An ileostomy was done by her surgeon, bypassing the entire colon. Neither resection of the colon nor dissection of the pelvis was attempted. The operative note described the peritoneal cavity as being free of any gross tumor. Approximately a year later she did have a new site of squamous cancer along the left labium minus. This appeared as a 1 cm ulcerative lesion, which was treated with an iridium 192 implant in December 1985. This lesion healed without evidence of persistent carcinoma. Whether this vulvar lesion was a recurrence of the earlier cervical primary or a new lesion was not clear. Although further follow-up failed to reveal any persistent disease, she also has been continued in the records as showing a partial response in this study. At the time of her last follow-up in July 1989, she had not received any further therapy and remained clinically free of apparent disease.

Patients 3, 4, and 5 (see Table I) experienced partial responses and also had no further treatment after completing this protocol therapy. Each patient has subse-

Table IIIA. Toxicity monitoring of combined interferon alfa and doxorubicin

Patient No.	*Hematologic parameters: Lowest recorded blood counts during treatment*		
	White blood cell count (No./mm^3)	*Granulocyte count (No./mm^3)*	*Platelet count (No./mm^3)*
1	5,200	5,148	339,000
2	5,300	3,551	197,000
3	4,700	2,021	201,000
4	1,500	900	349,000
5	5,200	4,270	334,000
6	4,500	3,375	332,000
7	4,900	2,989	333,000
8	11,400	10,032	697,000
9	4,000	2,320	313,000
10	4,700	3,478	595,000
11	3,300	2,178	169,000
12	2,400	1,392	193,000
13	3,400	2,346	220,000
14	4,500	2,790	326,000
15	9,000	6,700	282,000
16	2,400	1,728	392,000
17	4,100	5,940	392,000
18	6,400	3,968	440,000
19	11,100	11,560	484,000
20	4,700	4,089	191,000
21	9,900	8,019	322,000

Table IIIB. Toxicity monitoring of combined interferon alfa and doxorubicin

Patient No.	*Other parameters for toxicity monitoring: Highest recorded value during treatment*				
	Aspartate aminotransferase (U/l)	*Bilirubin (mg/dl) (total)*	*Blood urea nitrogen (mg/dl)*	*Nausea and vomiting**	*Fever†*
1	28	0.3	29	—	G2
2	9	0.3	11	G2	G2
3	28	0.9	25	G2	G2
4	13	0.4	15	G2	G2
5	24	0.6	15	G2	G2
6	43	0.7	13	G2	G3
7	10	0.6	10	G1	—
8	14	0.3	15	G1	—
9	93	0.9	9	—	—
10	10	0.5	9	G1	G2
11	38	0.5	10	G2	—
12	17	0.6	16	G2	G1
13	28	0.5	18	G1	G1
14	19	0.6	18	G2	G2
15	12	0.6	10	G1	G1
16	15	0.3	11	G3	G2
17	60	0.4	21	G2	G2
18	28	0.4	22	—	G2
19	21	0.3	17	G1	G2
20	45	0.5	24	—	—
21	18	0.4	20	G1	G2

*World Health Organization criteria: *G1,* Nausea; *G2,* transient vomiting; *G3,* vomiting requiring therapy.

†World Health Organization criteria: *G1,* fever <38° C; *G2,* fever 38° to 40° C; *G3,* fever >40° C; *G4,* fever with hypotension.

quently died, with survival durations of 9, 10, and 6 months, respectively. Patient 6 was on study from May to November 1985, initially experiencing a partial response of a measurable lung mass. Tumor progression was first seen in November 1985, at which time she had new chemotherapy started. The progression-free interval related to this study was 6 months.

The remaining patients had stabilization of disease for a period of time, followed by ultimate disease progression and death.

Bone marrow toxicity from this regimen was minimal (Tables IIIA and IIIB). Only one patient (No. 4) experienced granulocytopenia to levels <1000/mm^3. Thrombocytopenia was not seen in any patient from this group. There were no measured toxic effects on renal or hepatic function. One patient (No. 6) did experience grade III febrile morbidity. This was transient and resolved without sequelae. Another patient (No. 16) had grade III nausea and vomiting, which was also temporary.

Comment

Evaluation of this phase II study with a novel drug combination such as interferon alfa and doxorubicin raises at least three questions: (1) Is there clinical activity when a biologic response modifier is used together with a cytotoxic drug in the treatment of cervical cancer? (2) Are there patient factors that are associated with increased probability of good response? (3) Can preclinical studies be used to design clinical trials, not only in selection of drugs but also as a guide to optimal sequencing and dosing?

The first question regarding clinical activity is easy to answer in a superficial way. There does appear to be some effectiveness of combined interferon alfa and doxorubicin in six of the 17 evaluable patients who had advanced cervical cancers. Of particular note is the long duration of response seen in two of the patients, each of whom had failure of previous standard therapy. A more fundamental question is whether both drugs are necessary to achieve clinical responses. Data from the 1970s have been reported on patients with advanced cervical cancer who were treated with doxorubicin alone or in combination with other standard cytotoxic drugs.[9, 10] Clinical responses have been seen in <20% of patients, with median survival duration <4 months. The authors of these articles concluded that doxorubicin alone had little activity in the treatment of advanced squamous cell cancer of the cervix. There is one report from Yugoslavia in which topical and intralesional interferon alfa treatment for cancer of the cervix was used.[11] The drug preparation used in this particular report was not purified recombinant interferon alfa but a natural preparation. Some antitumor activity was reported in six of 15 patients, although clinical evaluation parameters were not always clear. There is no similar study of single-agent interferon alfa reported from any institution within the United States. We are left with the question whether the clinical responses seen have resulted from interferon alfa activity alone or whether there truly is some synergistic activity of interferon alfa plus doxorubicin (as in vitro studies suggest). A single-arm phase II trial such as this one will not be able to answer that question.

Historic experience with cytotoxic drugs and biologic response modifiers would suggest that there are patient factors that can be predictors of good responses. Good performance status, younger age, longer progression-free interval before relapse, complete responses from initial therapy, and smaller tumor burden all seem to be favorable prognostic features. In looking at these questions, we have divided our 17 evaluable patients into the six responders and the 11 nonresponders and looked at several of the parameters individually, with this treatment regimen (Table IV). Histologic cell type may be of significance. Each of the six responding patients had squamous tumor. None of the patients with adenocarcinoma or adenosquamous tumor showed measurable responses. Other host factors such as age, performance status, and duration of disease before entry into this study do not seem to be different between responders and nonresponders. All of the patients had tumor volume >1 cm.

Table IV. Patient factors correlated with treatment responses

Factor	*Average for responders*	*Average for nonresponders*	*Average for total group*
Age (yr)	52.5	46.5	48.6
Performance status	1	1.1	1.05
Duration of disease before entry in study (mo)	10.3	16.9	14.6

The question of drug delivery to tumor cells within a previously irradiated field is important to note. Interestingly, four of the six with response had evaluable disease within a previously irradiated field. Patients having only lung metastases (which was always outside the radiated field) showed responses in only one of four cases.

The last question concerns the application of preclinical studies as a guide in the design of clinical trials. Previous work has shown that certain in vitro tests such as a human tumor colony–forming assay can be used to predict antitumor activity of certain drugs.[12] Clinical correlations have been high when single drugs have been tested in vitro and compared with single-agent therapy in patients. However, no prospective trials have been done correlating in vitro human tumor colony–forming assay results with clinical use of doxorubicin or interferon alfa in the treatment of cervical cancers. We only know that cell lines that have been derived from human cervical cancers have shown additive and occasionally synergistic antiproliferative responses when treated with combined interferon alfa and doxorubicin.[3, 5]

Conclusions to be drawn include the observation that six of 17 evaluable patients with advanced or metastatic cancer of the cervix treated with sequential interferon alfa and doxorubicin have shown measurable clinical responses. Toxicity has been minimal, even in patients who have had pelvic radiation therapy. There does not appear to be a selection bias of only favorable patients being entered into this study. Looking to the future, preclinical data do seem to be useful in the design of phase II trials, particularly when there are only limited clinical parameters available on which to base new study designs.

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Discussion

DR. JAMES P. LAPOLLA, Tampa, Florida (Official Guest). The incidence of invasive cervical cancer, although declining, still accounts for 12,000 to 13,000 cases in the United States annually, with an expected 6000 to 7000 deaths. Currently, chemotherapy for recurrent disease is a less than satisfactory treatment modality, and newer agents or treatments with greater antineoplastic activity are needed. As we are aware, the most active single agent for the treatment of advanced or recurrent cervical cancer is cisplatin. The overall response rate to cisplatin averages about 25% for squamous cervical cancer, and the median survival of those responding is 9 months, on the basis of Gynecologic Oncology Group data.[1] Cisplatin-based combination therapy has not been demonstrated to improve survival over single-agent cisplatin to date.[2] Doxorubicin as a single agent for the treatment of advanced cervical squamous carcinoma has an overall response rate of 16% on the basis of a number of studies and would be classified as a drug with moderate to low activity for squamous cervical cancer.[1] Clinical trials using interferon for the management of advanced cervical squamous carcinoma are scarce. In 1981 Ikic et al.[3] using unpurified human interferon topically or topically and intramuscularly as a single agent reported that six of 15 patients with primary cervical squamous carcinoma manifested a response to treatment. In those responding to treatment five had no residual tumor and one had only residual microinvasion on the surgically resected specimens. Recently Kavanagh et al.[4] reported on the treatment of 25 patients with recurrent cervical squamous cancer treated with the combination of interferon alfa, 5-fluorouracil and cisplatin. They noted an overall response rate of 36% (9/25), similar to the rate in the current study, with two thirds of responding patients having a complete response. Survival data, however, were not reported. Welander et al. have presented to us a unique clinical trial in which patients with advanced or recurrent cervical carcinoma are treated on the basis of promising preclinical data suggesting that sequential treatment of cervical carcinoma cell lines with recombinant interferon alfa, then doxorubicin resulted in either synergistic or additive cell growth inhibition, compared with interferon alone, as demonstrated by in vitro chemotherapy testing with the human tumor clonogenic assay. Both the dose and scheduling of these drugs were based on in vitro data that suggested that interferon-induced cytotoxicity was related to maximum concentration achieved and to a prolonged exposure time. Doxorubicin treatment before interferon treatment did not result in a synergistic interaction, but after interferon exposure it did.[5]

Dr. Welander has previously reported to this association his work regarding the human tumor clonogenic assay for chemotherapy testing.[5] It was reported that for single-agent chemotherapy prospectively selected on the basis of the human tumor clonogenic assay, the "sensitive" predictive accuracy was 90% and the "resistant" predictive accuracy was 100%, indicating that the assay's prediction of response, or lack of response, to single-agent therapy was excellent. Other investigators who have correlated in vitro prediction with clinical response have found a "sensitive" predictive accuracy of 60% to 70% and a "resistant" predictive accuracy of 90%.[7] In this study 35% of cervical cancers were responsive to the interferon and doxorubicin combination chosen on the basis of human tumor clonogenic assay sensitivity testing. Although the predictive accuracy is less than expected for single agents considering the heterogeneity of cervical cancer cells, I think the results are quite remarkable. This response rate certainly is comparable to response rates observed with Cisplatin alone. It is also remarkable that two of six patients who did respond had prior cisplatin therapy. These results are promising, and larger phase II trials are certainly in order so as to gain more accurate information regarding the sensitivity of cervical squamous carcinoma to this combination-drug regimen.

Dr. Welander, you suggest that patient factors such as age, performance status, and disease duration before interferon-doxorubicin therapy did not differ for responders and nonresponders, so that patients clinically seemed comparable. However, for each patient treated in this study, were samples of each individual patient's tumor analyzed by the human tumor clonogenic assay for chemosensitivity to sequential interferon-doxorubicin therapy, and if so how did these results correlate with patient response?

Second, you have indicated in a previous report that the combination of interferon with cisplatin demonstrated additive effects on the human tumor clonogenic assay.[5] Have you done any trials using the three drugs—interferon, doxorubicin, and cisplatin—together, and if so are the results any better than with the two-drug interferon-doxorubicin protocol?

Finally, you previously indicated in 1983 that more experience would be necessary before you could recommend the human tumor clonogenic assay as a decision-making tool. In 1991 should clinicians use this assay for selecting chemotherapy for a patient with recurrent cervical malignancy?

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DR. EDWARD J. DENNIS, Columbia, South Carolina. About 8 years ago, in Columbia, South Carolina, in a 1-year period we had 50 cases of carcinoma of the cervix that were stage IIB or above. The average age of the patients was ≥55 years. We see advanced disease in patients with no previous therapy. Do you see any advantage, recognizing that there may already be positive periaortic nodes outside the field of radiation therapy, to using your chemotherapy regimen as initial therapy before or in combination with radiation treatment?

DR. WELANDER (Closing). We have done human tumor clonogenic assay sensitivity testing on a random basis but not as a formal part of this particular protocol. If we were in an ideal world, it would be our goal to do that. We would design a study that would prescreen patients by looking at their tumor drug-sensitivity pattern in advance. It would be easiest in patients who have not had radiation therapy. Laboratory findings suggest that it would be a good idea to combine interferon with one or more drugs, such as doxorubicin or cisplatin.

In Atlanta, for the bulky stage IB tumors that are >3 cm in diameter, we administer three weekly cycles of chemotherapy and then perform surgery. Although we have not done it, I believe this regimen could be used in advanced IIB or IIIB disease. Chemotherapy would then be followed by radiation therapy. It is a short course that does not significantly delay standard therapy and may help with outcome.

Primary invasive vaginal carcinoma

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A review of primary vaginal carcinoma treated at the Medical University of South Carolina from January 1970 through December 1989 included 76 squamous carcinomas, 12 adenocarcinomas, and 3 undifferentiated carcinomas. Staging was done according to the system of the International Federation of Gynecology and Obstetrics as modified by Perez et al. Stages I, II, III, and IV included 25, 39, 15, and 12 patients, respectively. Corrected 5-year survival rates were 73% for stage I, 39% for stage II, 38% for stage III, and 25% for stage IV. Sixteen percent of patients had received prior pelvic radiation. Invasive cervical cancer preceded vaginal cancer in 21% of patients. Detection of cancer was accomplished by routine cytologic testing in 17% of patients, palpation of an asymptomatic mass in 10% of patients, or palpation of a symptomatic mass in 72% of patients. Eighty-seven percent of patients were treated with radiation therapy. Survival curves of patients grouped by stage and other potential prognostic factors were compared. Lower stage ($p < 0.01$), younger age ($p < 0.02$), and no symptoms at detection ($p < 0.01$) were statistically significant favorable prognostic factors. Histologic type, extent of vaginal involvement, vaginal location, prior radiation therapy, prior cervical cancer, and prior hysterectomy are factors that did not significantly affect survival. (Am J Obstet Gynecol 1991;165:292-3.)

Key words: Vaginal cancer, prognostic factors, radiation therapy

Primary invasive carcinoma of the vagina accounts for 1% to 2% of all gynecologic malignancies.[1-5] As a result experience with this disease at any given institution is usually limited. Controversy remains concerning factors that significantly affect survival, appropriate staging of the disease, and ideal therapy.[5-8]

A report from the Medical University of South Carolina analyzed 31 cases of invasive carcinoma evaluated and treated from 1954 through December 1969. This report reviews the experience with 91 subsequent cases. Specific attention is placed on analysis of factors affecting survival.

Material and methods

The Tumor Registry at the Medical University of South Carolina was used to identify potential cases of primary vaginal carcinoma that were evaluated and treated at the Medical University of South Carolina from January 1970 through December 1989. Patients whose vaginal tumors also involved the vulva or cervix, who had carcinoma of the cervix or vulva within the preceding 5 years, or who had a history of endometrial carcinoma were excluded. The International Federation of Gynecology and Obstetrics (FIGO) staging system as modified by Perez et al.[9] was used prospectively. For all accepted cases it was possible to determine extent of disease from clinical records. Appropriate assignment of FIGO stage for each case was confirmed.

Available records included Tumor Registry patient abstracts, hospital charts, outpatient records, operative notes, and pathology reports. Patient follow-up was documented by the Tumor Registry and included records at the Medical University of South Carolina, as well as direct communication with patients and referring physicians.

Patient survival was plotted with the life-table method of Kaplan and Meier.[10] Potential risk factors influencing survival times of patients were examined by means of the Cox proportional hazards model.[11] Stepwise regression with the maximum partial likelihood ratio method was used to select the most significant factors. Survival curves were compared with the use of the log rank test.[12]

Results

Table I lists the number of patients with different gynecologic malignancies treated at the Medical University of South Carolina from January 1970 through December 1989. Ninety-one patients with primary vaginal carcinoma were treated. Seventy-six patients (84%) had epidermoid carcinoma, 12 patients (13%) had adenocarcinoma, and 3 patients (3%) had undifferentiated carcinoma. Table II lists these patients by FIGO stage modified as proposed by Perez et al.[9] Fifty patients

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were white and 41 were black. This is in agreement with the ethnic composition of our hospital population. The median age of patients was 60 years with a range of 21 to 88 years. Sixty-eight (75%) patients were at least 50 years old. Parity was known for 75 of our 91 patients. Seventeen patients (23%) were nulliparous and 26 (35%) were para ≥5.

Significant gynecologic events in the medical history are listed in Table III. For those patients who had a history of cervical cancer, the median interval between diagnosis of the cervical carcinoma and diagnosis of vaginal carcinoma was 168 months with a range of 68 to 338 months.

A means of detection could be identified in 87 of our 91 patients. Diagnosis was made by biopsy in all cases. This biopsy was prompted by a symptomatic mass in 63 patients, vaginal cytologic abnormalities in 15 patients, and an asymptomatic mass noted by an examiner in an additional 9 patients. For 85 patients it was possible to determine whether they had specific symptoms. This information is shown in Table IV. For 54 of 63 patients with symptoms it was possible to determine the duration of symptoms. The median duration of symptoms was 3 months. Thirty percent of patients had symptoms for ≤1 month, and only 22% of patients had symptoms for >6 months. A precise anatomic location of the tumor could be specified for 84 patients. The cancer was confined to the upper one third of the vagina in 33 patients, the middle one third in 5 patients, and the lower one third in 8 patients. The disease occupied the upper two thirds of the vagina in 16 patients, the lower two thirds of the vagina in 4 patients, and the entire vagina in 18 patients. In 11 of the 29 patients with prior hysterectomy, the disease was confined to the vaginal apex. The disease was located principally on the anterior vaginal wall in 14 patients and on the posterior vaginal wall in 10 patients. The right vaginal wall was the principal site in 15 patients, and the left vaginal wall was the principal site in an additional 18 patients.

The initial treatment of these 91 patients is detailed in Table V. The radiation therapy technique is described in Table VI. The sum of the teletherapy midplane tumor dose, brachytherapy vaginal rad surface dose, and interstitial radium mid tumor dose could be calculated for 76 of the 79 patients receiving radiation therapy. The median value was 80 Gy with a range from 16 to 121.7 Gy. Radiation therapy complications necessitating hospitalization occurred in 7 patients (9%). Transient proctitis occurred in 2 patients. An additional 2 patients had vulvar excoriation that required topical care and necessitated temporary interruption of therapy. One patient had a vulvar ulcer that required approximately 4 months to heal. One patient required a 4-day hospitalization and intravenous hydration for acute enteritis. A vesicovaginal fistula developed in a patient with bulky pelvic disease while she was receiving teletherapy.

Table I. Malignant neoplasms of female genital tract, 1970 to 1989, Medical University of South Carolina

Site	No. of patients	%
Cervix	1576	53.8
Uterine corpus	648	22.1
Ovary	469	16.0
Vulva	121	4.1
Vagina	91	3.1
Fallopian tube	24	0.8
TOTAL	2929	

Table II. Patient distribution according to FIGO (modified Perez) classification

Stage	No. of patients	%
I	25	27
IIA	17	19
IIB	22	24
III	15	16
IVA	6	7
IVB	6	7
TOTAL	91	

Exenterative procedures were used in 8 patients. Anterior exenteration was performed in 3 and 5 underwent total exenteration. Exenterative procedures were performed because of persistent disease after radiation therapy in 6 of 8 cases. There were no operative deaths. Only 3 patients died of persistent or recurrent vaginal cancer. Four patients have survived free of disease from 80 to 134 months. A rectovaginal fistula developed in one patient after anterior pelvic exenteration and postoperative intracavitary and external radiation therapy for stage IVA disease. She survived 253 months before dying of an unrelated cause.

Eleven patients received chemotherapy, ten of them because of a recurrence. Seven of these 11 patients received cyclophosphamide (Cytoxan) alone. One patient received melphalan (Alkeran). The remaining 3 patients received combinations containing doxorubicin (Adriamycin) and Cytoxan. No objective responses were noted.

A survival curve for all patients grouped by stage is shown in Fig. 1. Potential risk factors influencing survival times of patients were examined by means of the Cox proportional hazards model.[11] Stepwise regression with the maximum partial likelihood ratio method was used to select the most significant factors. Lower stage ($p < 0.01$), younger age ($p < 0.02$), and lack of symptoms at detection ($p < 0.01$) were identified as significant favorable prognostic factors. Anatomic location,

Table III. Significant preceding gynecologic conditions and treatment

Treatment or condition	*No. of patients*	*%*
Total hysterectomy	29	32
Cervical cancer	18	20
Pelvic radiation	15	16
Cervical intraepithelial neoplasia	6	7

Table IV. Symptoms of vaginal cancer in 85 patients

Symptom	*No. of patients*	*%*
Mass	63	74
Spontaneous bleeding	40	47
Urinary	19	22
Pain	13	15
Postcoital bleeding	7	8
Nonbloody discharge	5	6
Dyspareunia	2	2
None	23	27

extent of vaginal involvement, prior pelvic radiation therapy, prior cervical cancer, and prior hysterectomy were not identified as significant risk factors. Survival curves for patients with adenocarcinoma compared with those for patients with epidermoid carcinoma by log rank test showed no significant difference in survival curves ($p = 0.29$).[12] Comparison of survival curves in the 39 patients with FIGO stage II disease divided into groups IIA and IIB as suggested by Perez et al.[9] did not show a statistically significant difference ($p = 0.23$).

All 6 patients with stage IVB died of disease from 1 to 45 months after diagnosis. Three of 6 patients with stage IVA disease survived >5 years without recurrence. All 3 survivors underwent pelvic exenteration and received radiation therapy.

Comment

The treatment schema for vaginal cancer exhibited in this study is similar to that in many reports.[1, 2, 6, 8] Surgery alone was used in only 9% of patients and was principally confined to those with stage I or stage IIA disease. Radical surgery was not routinely performed for such patients as described in other reports.[7, 13] According to the recommendations of Rutledge,[1] Prempree et al.[14] and Perez and Camel,[8] even early lesions were treated principally with radiation therapy. Forty percent of our patients with stage I were treated with radiation therapy alone and an additional 40% were treated with a combination of radiation therapy and partial vaginectomy. Two thirds of our patients with stage II were treated with radiation therapy alone with an additional 20% treated with a combination of radiation therapy and surgery.

In spite of the recommendation by several authors that stage I and II lesions with a depth of >½ cm be treated with interstitial radiotherapy,[8, 14, 15] our report and others show that this modality has been infrequently used.[5, 6] Only 4 patients received interstitial radiation therapy in this series. Puthawala et al.[16] are leading proponents for the use of the Syed template for delivering interstitial radiation therapy to such patients. They reported recently on 16 patients with stage II treated with interstitial therapy by the Syed template. Local control was achieved in 14 patients. Twelve of 16 patients were free of disease with a medium follow-up of 50 months. This is considerably better than the 39% 5-year survival noted in our 39 patients with stage II. The potential advantage of interstitial radiation therapy for such patients is not unexpected. The relative intensity of radiation at two different points from a source of radiation is inversely proportional to the square of the distance of each point from the radiation source. With the placement of multiple interstitial radioactive sources, all areas of a large tumor can be close to the radiation source. With intracavitary radiation the vaginal surface is very close to the radiation source while areas of vaginal tumor may be significantly farther away. Thus extremely high vaginal surface doses would be required to produce modest doses deep within the vaginal tumor. Hintz et al.[17] have shown that the tolerance of the vagina is exceeded above 98 Gy in the lower vagina and 140 Gy in the upper vagina. With such relatively low tolerance by the vagina, it is not possible to deliver significant doses from an intravaginal source to vaginal tumors extending more than 1 to 2 cm from the vaginal surface. Nonetheless, much greater clinical experience will be needed to definitively establish the superiority of interstitial radiation therapy in the treatment of vaginal cancer.

Pelvic exenteration was used in 8 patients in our series. Seven patients received radiation therapy and underwent pelvic exenteration. This combined approach resulted in 5-year disease-free survival in 3 of 6 patients with stage IVA. Two other patients with stage IVA were treated with palliative radiation therapy and survived only 7 and 10 months. The remaining patient with stage IVA received palliative radiation therapy and underwent a diverting ileal urinary conduit but was lost to follow-up after 3 months. These results encourage an aggressive approach to stage IVA vaginal carcinoma and support the recommendation of initial radiation therapy followed by an appropriate exenterative procedure.

Our overall 5-year survival for patients with stage IV disease was only 25% because there were no survivors among the 6 patients with stage IVB disease. Dismal results were encountered in this group in spite of the fact that 3 of 6 patients had stage IVB disease on the basis of inguinal metastases with no other evidence of disease beyond the pelvis.

In this series no objective responses were noted in

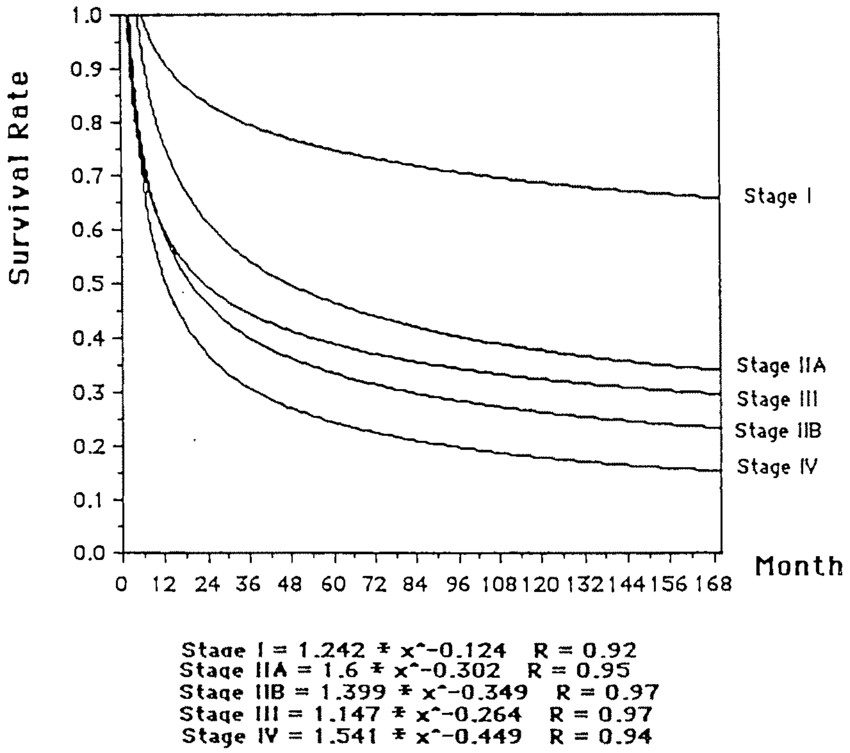

Fig. 1. Survival curves by FIGO staging.

Table V. Initial therapy given

Stage	*Radiation therapy*	*Radiation therapy and surgery*	*Surgery*	*Chemotherapy*	*None*	*Total*
I	10	10	5	0	0	25
II	26	8	2	1	2	39
III	15	0	0	0	0	15
IV	5	5	1	0	1	12
TOTAL	56	23	8	1	3	91

Table VI. Technique of radiation treatment used

	Stage			
Radiation therapy	*I*	*II*	*III*	*IV*
External	2	7	7	7
External and intracavitary	12	22	8	2
External and interstitial	1	3	0	0
External intracavitary and interstitial	0	0	0	1
Intracavitary	5	1	0	0
Interstitial	0	1	0	0
TOTAL	20	34	15	10

11 patients receiving alkylating agent chemotherapy. However, all but one of these patients received radiation therapy to the area of recurrence before receiving chemotherapy. It is of interest that a phase II trial of cisplatin in advanced or recurrent cancer of the vagina reported only one objective response in 16 patients with squamous cell carcinoma.[18] This response occurred in 1 of only 2 patients who had received no prior radiotherapy. A similar objective complete response with cisplatin was noted in a patient reported on by Holleboom et al.[19] with a metastatic site outside the radiation treatment port. Another case report by Katib et al.[20] describes a complete response achieved with bleomycin, methotrexate, and cisplatin in a patient with an 8 cm

vaginal mass and a 4 × 2 cm right inguinal metastasis. The patient subsequently received treatment with external and intracavitary radiation and at the time of the report had remained disease free for 7 months. From these reports there is a clear suggestion that chemotherapy is ineffective at sites previously treated with radiation therapy. This supports the use of cisplatin-containing chemotherapeutic regimens administered either concurrently or before radiation therapy. The use of radiation-sensitizing agents with cisplatin may offer the most promise for improved local and distant control of this disease. Given the rarity of this condition cooperative efforts testing this hypothesis would be most helpful.

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Discussion

DR. ROLLAND J. BARRETT II, Winston-Salem, North Carolina (Official Guest). Vaginal carcinoma continues to be one of the most difficult gynecologic malignancies to study, as well as to treat. Reports are limited by the relatively small number of patients treated at any one institution. Although the biologic behavior appears to be similar to that of carcinoma of the cervix, cure rates are generally lower in vaginal carcinoma. The most recent FIGO data show an overall cure rate of 54% for carcinoma of the cervix compared with only 39% for vaginal carcinoma.[1] Differences may be due to anatomic features that promote earlier distant spread in vaginal carcinoma, as well as to anatomic limitations to curative treatment. In spite of these difficulties cure rates have improved, primarily because of advances in radiotherapeutic techniques. The disease was essentially considered incurable in the mid 1930s. Survival has steadily improved since that time with this study noting a 46% overall survival. This report of 91 patients treated at the Medical University of South Carolina makes a significant contribution to the literature concerning primarily invasive vaginal carcinoma.

Common patient complaints at the time of initial presentation include pain, bleeding, and urinary difficulties. This study notes that 74% of patients first presented with a symptomatic pelvic mass. Of importance to all gynecologists is the fact that a significant number of cases of vaginal carcinoma are first detected when there are no symptoms but Papanicolaou smear is abnormal. Seventeen percent of cases in this study were detected by Papanicolaou smear alone, a finding similar to those of previous reports. The manuscript did not detail the cytologic diagnosis, but surprisingly 30 of 32 abnormal Papanicolaou smears in the study of Spirtos et al.[2] were interpreted as squamous dysplasia. This information on Papanicolaou smear diagnosis must be viewed in light of the occurrence of carcinoma of the vagina in patients with previous hysterectomy. In the report of Gallup et al.[3] of 28 patients and review of the literature, >40% of patients with carcinoma of the vagina had undergone hysterectomy primarily for benign indications. This study also reports a similar experience. Although previous hysterectomy appears to be a risk factor for vaginal carcinoma, in a recent case-control study reported by Herman et al.[4] matching patients for age and prior cervical disease, no increased risk was found in women undergoing hysterectomy because of benign disease. Using regression analysis Eddy et al. identified detection before symptoms as a favorable prognostic factor. I would first like to ask Dr. Eddy what his recommendations are for Papanicolaou smear screening for vaginal carcinoma in patients with previous hysterectomy in light of the findings reported.

Control of disease in the pelvis continues to be a problem in the treatment of vaginal carcinoma. Preventing local recurrence appears to be dependent on a combination of external therapy and brachytherapy in most patients with stage I or II disease. A midtumor or medial parametrial dose of 70 to 75 Gy may be necessary to achieve tumor control. Because of alterations in vaginal anatomy as a result of age, previous surgery, tumor mass, and previous irradiation, the placement of a vaginal cylinder for brachytherapy is often difficult. In many cases interstitial therapy allows adequate brachytherapy doses to be delivered. The improved overall local recurrence rate of 9% in the report by Spirtos et al.[2] compared with a 41% incidence reported by Peters et al.[5] must be due in part to the increased utilization of interstitial implants. These results appear to be contrary to Dr. Eddy's stated conclusions concerning interstitial radiation therapy. Second, I would like to ask if there is information from this series correlating radiation dose with local control.

Although there is no consensus as to the proper management of primary vaginal carcinoma, radiation therapy is considered the treatment of choice with surgery relegated to a limited role. In reporting the University of Pennsylvania experience with 75 cases of primary squamous cell carcinoma of the vagina, Rubin et al.[6] described 15 patients undergoing radical pelvic surgery with intent to cure. Of particular note is the fact that only 1 of 7 patients undergoing some type of exenterative procedure was alive after 5 years of follow-up. This study is more encouraging concerning the role of pelvic exenteration, with a significantly higher survival rate in patients with stage IV compared with results of most other studies. My third point is to ask Dr. Eddy to elaborate on his recommendation of initial radiation therapy before the appropriate exenterative procedure in patients with stage IVA. In a related question, how were patients with early-stage disease and prior radiation therapy for cervical cancer treated?

Improving survival in patients with vaginal carcinoma will also depend on controlling metastatic disease and extrapelvic failure. This series confirms the present lack of effective chemotherapeutic agents for metastatic squamous cell carcinomas of the lower genital tract. Concomitant radiation therapy and chemotherapy with cisplatin regimens holds promise for improving local control.[7] A reduction in the incidence of extrapelvic recurrence is less certain. Although large cooperative efforts involving many institutions are needed to study this relatively rare malignancy, it is discouraging to note that the Gynecologic Oncology Group has recently been forced to terminate studies of vaginal carcinoma because of difficulties in patient accrual and timely completion of proposed studies.

In spite of these limitations continued study is necessary. This report from the Medical University of South Carolina is one of the largest series reported to date and a welcome addition to the literature. I congratulate Dr. Eddy and his colleagues for this important contribution.

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Dr. Eddy (Closing). I believe that all women should have annual Papanicolaou smears, particularly those who have had a hysterectomy. That is because of the ease of performance, the low cost of the test, and the fact that patients who had a hysterectomy made up 32% of our patients with vaginal cancer. Patients who had malignancy detected by Papanicolaou smears were more likely to have microinvasive or stage I vaginal cancer, and they clearly had a better outcome than the other patients. I have spent a lot of time trying to determine how radiation dose correlated with local cancer control. Another study is under way in an attempt to look at this question in depth. All studies are flawed by relatively small numbers of patients. When you break them into subgroups, you get such small numbers of patients that most statistical tests are invalid. We have found that patients who had <55 Gy did so poorly that they significantly affected the survival rate of any group in which they were included. One reason that some patients received <55 Gy is that they died during therapy or they could not have intracavitary radium for any one of a variety of reasons. Therefore looking at absolute numbers creates difficulties. The other problem is in the number that we all quote as "midtumor dosage" or "total midtumor dosage." Both are numbers that have been invented. It is not fair to add external radiation midtumor doses to radiation surface doses and look at the total dosage. Clearly, if the total dosage were delivered by external beam, it would be significantly more powerful and probably would kill all of our patients. There is a great deal of difficulty in looking at raw numbers.

I have been asked to elaborate on our recommendation for radiation therapy before exenterative procedures in patients with Stage IV. It was remarkable that three patients with stage IVA who were treated with radiation were noted to have persistent disease at exenteration and all were cured. I think that this is probably due to good fortune. It was noteworthy that every patient treated for palliation didn't get very much and lived a relatively short period of time. This makes us more interested in being aggressive in considering exenteration in these patients. Another interesting subgroup are those with stage IVB, all of whom died in relatively short periods of time. They also received external therapy, as it was most difficult to give intracavitary therapy for "palliation." Three of these patients were classified as having stage IV disease, only on the basis of positive inguinal nodes. I am tempted to recommend groin dissection and radiation therapy for such patients with consideration for exenteration in patients with persistent central disease. I know that this recommendation is controversial and not based on fact.

How are patients with early-stage disease and prior radiation therapy treated? We were fortunate in that most of our early-stage disease was stage I; some of these patients had microinvasive cancer that was diagnosed after colpectomy. Four of eight such patients were treated by colpectomy alone. Two patients were treated by external radiation therapy, one by external beam therapy and colpectomy, and one by intracavitary radium and external beam therapy. Those eight patients had local control of disease as a result of the therapy.

Analysis of 500 obstetric and gynecologic malpractice claims: Causes and prevention

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Five hundred obstetric and gynecologic malpractice litigation claims were analyzed to determine the incidence of claims that could not be defended because of breach of standards of care, problems with documentation, or both. Of the 500 claims, 294 were obstetric and 206 were gynecologic. Of the obstetric claims, 79 (27%) were indefensible because of breaches of standards of care (71%), problems with documentation (5%), or both (24%). Of the 206 gynecologic claims, 45 (22%) were indefensible because of breaches of standards of care (62%), problems with documentation (24%), or both (13%). The incidence of indefensible obstetric and gynecologic claims was 25%. Indefensible claims were analyzed to determine the common medical and surgical problems that instigated the malpractice suits. Recommendations are made that should reduce the incidence of indefensible malpractice suits. (AM J OBSTET GYNECOL 1991;165:298-306.)

Key words: Breach of standards of care, documentation, malpractice

The malpractice crisis has had significant impact on obstetricians, gynecologists, and the women who seek their services.

The 1990 American College of Obstetricians and Gynecologists (ACOG) Professional Liability Survey[1] revealed that 78% of obstetricians and gynecologists have been sued at least once; 37% have been sued three or more times. The survey also revealed that 62% stopped practicing obstetrics before the age of 55 years and that 31% discontinued obstetrics before the age of 45 years. Twenty-four percent of obstetricians have decreased their level of high-risk obstetrics.

Another survey[2] revealed that 36% of obstetric residents have been sued while in training.

The 1990 ACOG survey[1] noted that obstetric malpractice claims outnumbered gynecologic malpractice claims by 58% to 42%. In this study of 500 claims the

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Table I. Analysis of 500 obstetric and gynecologic malpractice claims

				Reason indefensible						
				Breach of standards		*Lack of documentation*		*Combination*		
	No. of claims	*No. defensible*	*No indefensible*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*% Indefensible*
Obstetric	294	215	79	56	71	4	5	19	24	27
Gynecologic	206	161	45	28	62	11	24	6	13	22
TOTAL	500	376	124	84	68	15	12	25	20	25

incidence of obstetric claims was 59% and gynecologic claims 41%.

I reviewed 500 obstetric and gynecologic claims to determine the causes of the claims and the percentage of indefensible claims. The indefensible claims were further analyzed to determine whether they were indefensible because of a breach in the standards of care, lack of documentation, or both. Recommendations are made that may significantly reduce further claims.

Material and methods

Between 1983 and 1988, I reviewed 500 obstetric and gynecologic malpractice claims primarily from the southeastern United States. The 500 consecutive cases, which were supplied by several insurance companies and attorneys, are analyzed in this report.

A case was categorized as indefensible when the treatment rendered was considered to be below the degree of care generally exercised by physicians under the same or similar circumstances. This standard of care was established after consultation of the following sources and references: (1) ACOG's *Standards for Obstetric-Gynecologic Services*, editions 1 through 6; (2) *AGOC Technical Bulletins*, 1968 through 1988; (3) articles indexed in the National Library of Medicine's MEDLARS database; and (4) *ACOG Committee Opinions*, Nos. 11 through 64.

Deviation from these standards without reasonable explanation was classified as being indefensible. Methods of therapy and procedures that might be controversial but were documented as acceptable through literature searches were classified as defensible and not malpractice. A poor outcome, or "maloccurrence," was never classified as malpractice unless it resulted from a direct deviation from the established standards. In this review substandard care per se did not necessarily render a case indefensible unless a cause-and-effect relationship existed between the substandard care and the poor outcome.

Results

The 500 claims were divided into 294 obstetric claims and 206 gynecologic claims (Table I).

Of the obstetric claims, 79 (27%) were classified as indefensible. Of these, 56 (71%) breached the standards of care, 4 (5%) had inadequate documentation, and 19 (24%) had both problems.

Of the gynecologic claims, 45 (22%) were classified as indefensible. Of these, 28 (62%) breached the standards of care, 11 (24%) had inadequate documentation, and 6 (13%) had both deficiencies.

Obstetric cases. Many of the obstetric claims comprised several clinical problems, making classification difficult. These cases were categorized according to the problem that instigated the malpractice suit (Tables IIA through IID).

Several problems common to the indefensible obstetric claims involved cord-placental problems, stillbirths, presence of meconium, fetal distress, dystocia of labor, abnormal presentations other than breech, cephalopelvic disproportion, brain damage, perinatal deaths, infant trauma, and vaginal birth after cesarean section. These claims could not be defended because of failure to (1) appropriately monitor or record the fetal heart rate with auscultation or electronic fetal heart monitoring, (2) recognize and act on potentially ominous fetal heart rate tracings, (3) recognize and appropriately manage dystocia of labor, (4) administer oxytocin according to ACOG guidelines, (5) have cesarean capabilities within 30 minutes without a reasonable explanation for the delay, (6) properly resuscitate the newborn, (7) respond to nurses' calls in a timely and appropriate manner when notified of potential problems, or (8) notify a physician when a nurse midwife encountered a high-risk situation during labor.

The postmaturity claims that could not be defended were caused by problems related to the use and misuse of antepartum fetal surveillance.

The indefensible cases of shoulder dystocia were so classified because the obstetricians ignored the risk factors of maternal diabetes, obesity, and fetal macrosomia coupled with dystocia of labor.

Breech deliveries were judged to be indefensible when there was improper use of oxytocin and failure to recognize dysfunctional labor.

In the indefensible forceps–vacuum extractor cases, physicians failed to evaluate and recognize dystocia of labor and attempted midforceps procedures that resulted in significant infant morbidity.

Table IIA. Analysis of obstetric cases: Prenatal labor and delivery

Cause	*No. of claims*	*No. defensible*	*No. indefensible*	*Reason indefensible*		
				Breach of standards	*Lack of documentation*	*Combination*
Cord-placenta problems						
Abruptio placentae	10	9	1			1
Prolapse of cord	5	4	1			1
Cord accidents	3	3	0			
Placenta problems	1	1	0			
Stillborn	14	9	5	5		
Problems involving meconium	13	7	6	4	1	1
Postmaturity	11	5	6	5		1
Shoulder dystocia	11	8	3	2		1
Premature rupture of membranes	10	10	0			
Breech	10	4	6	5		1
Fetal distress	9	4	5	5		
Forceps–vacuum extractors	8	5	3	3		
Prematurity	7	7	0			
Pregnancy-induced hypertension	7	5	2	1		1
Oxytocin	6	0	6	4		2
Twins	5	5	0			
Miscellaneous delivery problems	5	5	0			
Anesthesia	5	5	0			
Dystocia of labor	3	1	2	1		1
Chorioamnionitis	2	2	0			
Abnormal presentation	2	1	1			1
Cephalopelvic disproportion	1	1	1			
Premature labor	1	1	0			

Table IIB. Analysis of obstetric cases: Problems in newborn

Cause	*No. of claims*	*No. defensible*	*No. indefensible*	*Reason indefensible*		
				Breach of standards	*Lack of documentation*	*Combination*
Brain damage	24	16	8	4		4
Congenital anomalies	9	8	1	1		
Neonatal seizures	6	6	0			
Circumcision	6	2	4	3	1	
Infections in newborn						
Herpes	3	3	0			
Miscellaneous	3	3	0			
Perinatal death	3	2	1		1	
Infant trauma	3	2	1			1
Respiratory distress	2	2	0			

When nurses failed to notify the physician or the physician failed to respond in a timely and appropriate manner to the obvious signs and symptoms of pregnancy-induced hypertension, the claims could not be defended.

Failure to diagnose a major congenital anomaly on a midpregnancy ultrasonogram by a physician who implied he possessed such diagnostic skills rendered one claim indefensible.

Circumcision claims became indefensible when electrocautery machines were improperly used or proper consent forms had not been signed.

In cases of abortion, indefensible claims arose from physicians' failure to adequately inform patients as to the signs and symptoms of infection, failure to recognize and appropriately treat the postoperative complications of infection resulting in serious morbidity and extensive surgery, and allowing physician's assistants to manage serious complications without adequate physician supervision.

Maternal death claims could not be defended because the physicians failed to recognize and appropriately treat sepsis; administer a general anesthetic and monitor the patient properly, resulting in an unrecognized gastric aspiration; administer oxytocin properly, resulting in hyperstimulation, abruptio placentae, and disseminated intravascular coagulation; and deliver a known high-risk obstetric patient in a hospital setting (the patient was delivered in a birthing center).

Claims related to x-ray examinations and drugs taken

Table IIC. Analysis of obstetric cases: Surgical problems

				Reason indefensible		
Cause	*No. of claims*	*No. defensible*	*No. indefensible*	*Breach of standards*	*Lack of documentation*	*Combination*
Abortion	25	22	3	1		2
Postpartum hemorrhage	5	5	0			
Cesarean section	4	2	2	1	1	
Bladder injuries	3	2	1			1
Postoperative infections	3	3	0			
Rupture of uterus	3	3	0			
Surgery in pregnancy	3	3	0			
Vaginal birth after cesarean section	2	1	1	1		
Episiotomy complications	2	2	0			

Table IID. Analysis of obstetric cases: Medical problems

				Reason indefensible		
Cause	*No. of claims*	*No. defensible*	*No. indefensible*	*Breach of standards*	*Lack of documentation*	*Combination*
Maternal deaths	13	7	6	6		
X-ray examinations and drugs in pregnancy	14	10	4	4		
Miscellaneous	5	5	0			
Amniotic fluid embolism	2	2	0			
Postpartum cerebrovascular accident	1	1	0			
Disseminated intravascular coagulation defect	1	1	0			

during pregnancy were classified as indefensible when there was no evidence that the physician had attempted to ascertain whether the patient was pregnant before ordering x-ray examinations or prescribing medications contraindicated in pregnancy.

Gynecologic cases. The leading gynecologic claim (Tables IIIA and IIIB) was unsatisfactory sterilization. When a physician failed to carry out a recognized surgical sterilization procedure properly and the patient became pregnant, the claim could not be defended. Failure to obtain signed consent forms for sterilization also contributed to indefensible suits.

Although injury to the genitourinary tract was the second major gynecologic malpractice claim, it accounted for the highest number of indefensible gynecologic claims. The claims were indefensible not because of the original injury but because the physician failed to recognize the signs and symptoms of the injury and therefore failed to bring about a timely repair of the injury, leading to permanent damage in two cases. Three of the claims were rendered indefensible because of lack of documentation in either the office or hospital records to justify the original surgery.

When a nurse failed to notify a physician of signs and symptoms of wound infection, or the physician failed to respond in a timely manner, the claim was judged indefensible. In one postoperative complication case, an irrigation needle was lost in the abdomen during a microsurgical procedure, resulting in an injury to the bowel and a need for further surgery.

The indefensible ectopic pregnancy claims resulted from several different problems. When the patient was initially seen with the classic signs of ectopic pregnancy and an appropriate investigation was not conducted, the case was deemed malpractice. When an elective abortion was attempted, and the tissue obtained was not diagnostic of an intrauterine gestation, and no further evaluation was conducted, the claim could not be defended.

A prolonged vaginal procedure, during which the patient was unfavorably positioned in the stirrups, resulted in bilateral foot drop and an indefensible claim of nerve injury.

Intrauterine contraceptive device (IUD) claims that could not be defended involved insertion of an IUD even though it was contraindicated for medical reasons, failure to investigate a lost IUD string, and failure to explain the potential risks of using an IUD.

Failure to diagnose breast cancer has become a more frequent claim. The cases in this series could not be defended because of physician failure to evaluate obvious breast masses with mammogram, surgical con-

Table IIIA. Analysis of gynecologic cases: Surgical

Cause	*No. of claims*	*No. defensible*	*No. indefensible*	*Reason indefensible*		
				Breach of standards	*Lack of documentation*	*Combination*
Sterilization	30	26	4		3	1
Injury to genitourinary tract						
Ureter	11	8	3	2		1
Vesicovaginal fistula	9	7	2	1	1	
Urethra	4	3	1		1	
Bladder	3	2	1		1	
Postoperative complications						
Wound infection	8	6	2		1	1
Foreign body	6	2	4	1	3	
Death	3	3	0			
Wound dehiscence	2	2	0			
Adhesions	2	2	0			
Miscellaneous	1	1	0			
Ectopic pregnancy	17	14	3	1		2
Laparoscopy complications						
Bowel injury	8	5	3	3		
Miscellaneous	7	7	0			
Curettage	11	11	0			
Nerve injury	6	5	1	1		
Appendicitis	5	5	0			
Rectovaginal fistula	5	5	0			
Bowel injury	3	1	2	2		
Miscellaneous surgical problems	18	14	4	3	1	

sultation, or both; failure to follow up on a suspicious mammogram report; and failure to perform a breast examination during the patient's annual physical examination.

The improper management of cervical pathologic findings noted on Papanicolaou smears led to claims of malpractice. When the pathologist suggested or the findings of the Papanicolaou smear warranted colposcopic examination and it was not performed, the defense of invasive cancers of the cervix was impossible.

The miscellaneous indefensible medical problem involved the misfiling of a laboratory report by office personnel. The physician did not discover that a patient had had positive test results for gonorrhea until after a full-blown case of pelvic inflammatory disease developed. The physician was held liable for the error.

Comment

This review confirms that documentation is a significant problem in medical recordkeeping. Twenty-four percent of the gynecologic cases and 5% of the obstetric cases were found indefensible solely because of failure to document records in a complete and contemporaneous manner. Failure to document records adequately was an important factor in another 24% of obstetric cases and 13% of gynecologic cases (Table I). In a courtroom the finest care rendered under the best circumstances may be difficult or impossible to defend if it is not documented. An altered record also will make a case impossible to defend. In front of a jury a physician's credibility can easily be destroyed.

When faced with a difficult obstetric or gynecologic problem, a physician should write out a plan of therapy or get consultation. It is easier to defend the judgment of two physicians even if the outcome is bad. Juries generally accept such judgments over those of experts who never attended the patient. It must be stressed that judgmental decisions, even if they result in maloccurrence, are defensible and do not constitute malpractice.

Several specific areas in the obstetric cases need to be addressed. Oxytocin was frequently a primary or contributing factor in the obstetric malpractice claims. The *ACOG Standards for Obstetric-Gynecologic Services* and the *ACOG Technical Bulletin* No. 110, offer quite specific guidelines that, if followed, would have made many claims defensible.

At its best, electronic fetal heart monitoring has serious limitations.[3-5] In this study the major problems relating to the electronic fetal heart monitor were failure to recognize and properly classify decelerations, respond to calls from nurses concerning potentially ominous patterns, form a resonable management plan when potentially ominous patterns were observed, and obtain a reasonably acceptable or readable tracing.

Two cases in this retrospective analysis were claims involving infants with neurologic impairments that were successfully defended by evidence gained through examination of the cord and placenta. Recent research[6]

Table IIIB. Analysis of gynecologic cases: Medical

Cause	No. of claims	No. defensible	No. indefensible	Reason indefensible: Breach of standards	Reason indefensible: Lack of documentation	Reason indefensible: Combination
IUD	11	6	5	5		
Failure to diagnose breast cancer	9	5	4	4		
Pelvic inflammatory disease	7	5	2	1		1
Management of cervical pathologic findings	3	1	2	2		
Management of endometriosis	3	3	0			
Medical errors	2	1	1	1		
Management of human papillomavirus infections	1	1	0			
Miscellaneous medical problems	11	10	1	1		

has demonstrated that neurologic impairment in an infant is not prima facie evidence of substandard care or negligence by the obstetrician at birth. Rather, pathologic conditions of the cord and placenta are increasingly recognized as the major cause of neurologic impairment.[7] These conditions often develop during the prenatal period long before labor and delivery; although they cannot be prevented by the best of obstetric care, they can be identified by examining the placenta.

If there is a maternal, fetal-newborn, or placenta–umbilical cord condition that warrants study (Table IV), the attending physician should arrange for a placental examination. If the pathologist is not well versed in placental pathology, he or she can still help by recording measurements, taking photographs, and making slides that can be reviewed by a placental pathologist. Placental examination often reveals the cause of preterm labor, premature rupture of the membranes, intrauterine growth retardation, and antenatal hypoxia.

Failure to recognize dystocia of labor was a complicating factor in several indefensible claims. Labor records that use the Friedman curve[8] would facilitate early recognition of the problem.

Before medication contraindicated in pregnancy is prescribed or x-ray studies to evaluate gastrointestinal complaints are ordered, it is sensible to at least list the patient's last menstrual period. If a question of pregnancy exists, it is imperative to test the patient for pregnancy before taking further action.

Sterilization failures accounted for the largest number of gynecologic malpractice claims. The majority of these claims might have been prevented if the physician had given the patient printed material, such as an ACOG pamphlet on sterilization, that outlines the risks, complications, and failure rate of sterilization procedures. It should be stressed that pregnancy can occur after the procedure, and it should be documented in the patient's chart that this information was given and discussed.

Table IV. Indications for placental examination

- Maternal
  - Diabetes mellitus
  - Pregnancy-induced hypertension
  - Premature rupture of the membranes
  - Preterm delivery (<36 weeks)
  - Postterm delivery (>42 weeks)
  - Unexplained fever
  - Poor obstetric history
  - History of drug use or abuse
- Fetus-newborn
  - Stillborn
  - Neonatal death
  - Multiple gestation
  - Intrauterine growth retardation
  - Congenital anomalies
  - Erythroblastosis fetalis
  - Transfer to neonatal intensive care unit
  - Ominous fetal heart tracing
  - Presence of meconium
  - Apgar score <5 at 1 min; <7 at 5 min
- Placental–umbilical cord
  - Infarction
  - Abruptio placentae
  - Vasa previa
  - Placenta previa
  - Abnormal calcification
  - Abnormal appearance of placenta or umbilical cord

Injuries to the genitourinary tract accounted for the second highest number of gynecologic cases; more important, they accounted for the highest number of indefensible cases. Injury to the ureter or bladder is an acknowledged risk of obstetric or gynecologic surgery. The frequency of ureteral injuries has been quoted by various authors to range from 0.1% to 30%.[9, 10] In this review the indefensible claims were caused by physician failure to respond in a timely and appropriate manner to the patient's signs and symptoms. When a surgeon promptly undertakes an evaluation of a suspected injury to the lower genitourinary tract, the defense is generally quite easy.

With the increased incidence of ectopic pregnancy in the last decade and the considerable maternal morbidity and mortality that accompany it, physicians must keep this diagnosis in the forefront of their differential diagnoses when confronted with a patient with abdominal pain, amenorrhea, and abnormal vaginal bleeding. The refinement of both urinary and serum pregnancy tests, coupled with the improvement in diagnostic ultrasonography, will aid in early diagnosis. Weckstein et al.[11] achieved an unruptured ectopic diagnostic rate of 27% with this approach.

Failure to diagnose breast cancer has recently become a rising cause of malpractice claims. Because many women consider their obstetrician-gynecologists to be their primary care physicians, a structured management plan is recommended. Breast cancer develops in 1 in 11 women[12]; the incidence of this disease is almost twice that of all gynecologic cancers combined. Physicians should encourage and teach patients breast self-examination at an early age. In this study breast cancer developed in four patients under 35 years of age and in one at 25 years of age.

The American Cancer Society[13] has made the following recommendations with regard to mammography: a baseline examination at 35 years of age, an examination every 2 years in the 40s, and annual examinations from 50 years of age. When a discrete mass or change in the breast is found by the patient or her physician, it is appropriate to reexamine the patient after her next menstrual period. If the suspicious area is still present, a mammogram should be taken. Even if the mammogram is normal, the patient should be referred for surgical consultation because the true-positive rate for mammography varies from 82% to 95.7%, according to studies by Holland et al.[14] This protocol makes claims for failure to diagnose breast cancer very defensible.

Human papillomavirus is not considered to be a likely cause of cervical cancer,[15] even though Koch's postulates have not been fulfilled. The early identification and eradication of infections with this virus should result in a decrease in cervical cancer. Colposcopic evaluation[16] of the cervix and lower genital tract in cases of abnormal Papanicolaou smears has proved to be beneficial to patients.

In conclusion, this analysis of 500 obstetric and gynecologic malpractice claims found an overall incidence of malpractice of 25%. This study did not confirm the popular public opinion that malpractice occurs because of a few bad physicians. Rather, a varied group of physicians, from professors at universities to board-certified obstetricians and gynecologists practicing a modern, well-equipped centers to physicians in rural level I hospitals, were all involved in malpractice claims. There were very few repeat offenders. Had the guidelines discussed in this study been followed, the majority of indefensible cases would have been defensible and the unfavorable outcomes might have been prevented.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. WILLIAM H. KIRKLEY, Fort Lauderdale, Florida. I feel a bit uneasy when someone states that a case is indefensible. There are too many variables, such as the defending attorney (extremely important), the quality of the records, and the defendant (will he or she make a good witness?). Many cases are settled because the defense attorney realizes it would be a disaster to place the defendant on the stand. Last, but not the only factor, is what happened. (I agree that if during circumcision you burn the head of the penis off, this is indefensible.) I read a report published by an insurance carrier that had several hundred cases reviewed by obstetrician-gynecologists in an academic setting. They found that malpractice occurred in >70% of the cases. Dr. Ward found 27% in obstetrics and 22% in gynecology. In several hundred cases reviewed I believed 10% to be indefensible. (I asked Dr. Ward if he could get 25 cases he believed to be indefensible and let me review them. Unfortunately, this was not possible.) The hardest decisions I have had to make involved whether to testify on behalf of the defendant in cases that I believed not to have been handled properly but that I believed to be defensible. I am sure everyone agrees that the expert witness's opinion is not necessarily the generally accepted standard of care. Dr. Ward used ACOG's *Standards*, with which we are all familiar. This makes me very nervous! Please let's not have a format or outline so that any deviation is detectable as malpractice by an attorney. ACOG never intended, and should not have, for this to be *the* standard of care; it is *a* standard of care. On numerous occasions I have testified that ACOG bulletins are merely the opinion of the four or five people on that committee. If you posed the circumstances of a trial to the 20,000 to 30,000 members of ACOG who actually establish the standard of care in their localities, you would get a diversity of opinions. Never let an article, book, or bulletin establish the standard of care (all ACOG publications carry this disclaimer).

I agree with Dr. Ward that poor records are a major problem. I believe they are *the* major problem. He noted that the records were so poor in 5% of the obstetrics cases that this alone mandated settlement; in 24% poor records were a strong contributing factor to the resolution. In gynecology 24% of the physicians failed to keep adequate records; in 13% inadequate records contributed to the outcome. Remember, even in the cases that go to trial, poor records may be a problem in your defense. George Hart of Atlanta, probably the best defending attorney in the country in cases involving brain damage, told me that the physicians in 75% of his cases had serious record-keeping problems.

Dr. Ward superifically passes over some problems that I am unable to comment on. I am sure time was a factor, but he probably couldn't be too specific because some cases may still be in litigation.

X-ray examinations and drugs taken during pregnancy should in general not be too hard to defend because they almost never cause a problem.

With ultrasonography and β-subunit human chorionic gonadotropin, ectopic pregnancy can be detected earlier. Failure to do so would be hard to defend, but not impossible.

If failure to investigate a lost IUD string is malpractice, I am guilty.

Contrary to Dr. Ward's findings in these 500 cases, missed breast cancer is the leading claim against gynecologists. If a simple protocol is adhered to, 99% of these cases will be eliminated. I presented this protocol at a District IV malpractice seminar that Gluck and I conducted in Baltimore 5 to 6 years ago. Had this protocol been followed, there would not be a problem today.

Does Dr. Ward believe it is malpractice to treat suspected or documented pelvic inflammatory disease without tuboovarian abscess on an outpatient basis, or does he believe that all patients should be admitted to the hosptial for thorough workups and be given multiple intravenous antibiotic therapy. Would the fact that the woman did not want children alter his opinion?

I have observed mismanagement of carcinoma of the cervix. One case I was involved in was amazing. For 2 years in a row an abnormal Papanicolaou smear, reported to be class II, "probably inflammatory," was ignored. The third year a lesion of the cervix was recorded. The fourth year advanced carcinoma of the cervix was observed. With so many physicians doing colposcopy, I am afraid that missed cervical cancer will be the lawyers' next big push.

Dr. Ward correctly advises us not to alter records. With duplicating machines widely available, you haven't a chance. I am amazed that I still see this happening. The most recent case was detected because the defendant's office personnel had sent records to the plaintiff's attorney before the chart was altered. Good communication with the patient and the family is important, as Dr. Ward points out very clearly. But, handle it properly. Be sure a nurse is with you; preferably talk with the patient and only one family member. After you have completed this talk, immediately write a note stating exactly what you told the patient and have the nurse cosign the note. The 10 or 15 minutes it takes to write this note may save you many hours in the courtroom and millions of dollars.

I believe it is difficult to be so dogmatic about so-called indefensible claims of error in monitoring and interpreting fetal tracings. I remember that 8 to 10 years ago 12 to 15 physicians were asked to review a number of electronic fetal heart monitor strips and were asked to give a diagnosis and prognosis. They agreed in only two or three cases! If the experts can't agree, how can we be so critical as to accuse a person of malpractice from a tracing. I strongly suggest that when an electronic fetal heart monitor strip is abnormal, note your awareness of this, your interpretation of it, and your course of treatment. Better still, as Dr. Ward suggests, have another physician consult with you and document the consultation.

I am surprised that improper management of a dystocia was an indefensible problem. I thought that at any suggestion of dystocia nowadays a cesarean section is performed. I would ask Dr. Ward if this was not done.

Postmaturity syndrome is so controversial and the protocol is so varied from institution to institution that I do not believe that the majority of the 15,000 to 20,000 ACOG members, particularly those practicing in small communities at level I facilities, will agree with the management proposed by our academic community.

As for the prolonged vaginal procedure during which the patient was unfavorably positioned in stirrups that resulted in foot drop, I ask Dr. Ward how he arrived at the idea of an unfavorable position. Or was the attending physician crazier than I think he was? This brings up a very important point: Never tell a patient or plaintiff's attorney you did wrong. I remember a physician doing this when he had done nothing wrong.

Dr. Alton V. Hallum, Jr., Atlanta, Georgia. How do you educate your pathologists who are not up to date, and how do you use those slides at a later date?

Dr. Murray A. Freedman, Augusta, Georgia. It is sad that we continue to treat the things that result from the liability crisis—rather than the cause, which lies in the fact that it is so terribly lucrative to one other profession.

Dr. Mary E. Blanchard, Sumter, South Carolina. We can tell all the lawyer jokes that we want to, but as Pogo says, "We have met the enemy, and the enemy is us." No lawyer can get any medical malpractice case to court at any time without a medical expert. As long as we have medical experts stating that the patient had an enema and that this caused the baby to be brain damaged, we are in deep trouble. I have been involved in litigation in which there were two experts from two "outstanding institutions" in this country. I called the chairperson of a department and he more or less told me that he was hired at that university with the proviso that a certain physician could continue to testify about anything that he wanted to testify about. The second physician, a friend of mine at a major institution, said that he felt bad that a member of his department was saying what he was saying. Sooner or later we have to take a stand with these people. Unless we do, we will continue to find ourselves in the crisis we are in.

Dr. Hugo S. Moreno, East Point, Georgia. Considering that the National Data Bank requests that any award or settlement above $100,000 be registered in the physician's name, do you think that this will increase the number of frivolous suits that go to court regardless of merit?

Dr. J. Greg Johnson, Greenville, South Carolina. Do you think that umbilical cord gas pH is an acceptable substitute for placental pathology because it is difficult to get pathologists to look at the placenta? What do you think of the routine use of umbilical cord gas pH?

Dr. Ward (Closing). I encourage the use of umbilical cord blood gas studies in all cesarean deliveries and in those infants delivered vaginally who have low Apgar scores. Umbilical cord blood gas studies do not replace placental examinations. One method of educating pathologists is to give them copies of the article by Naeye (see reference 6 of article). If pathologists are not knowledgeable about the placenta, they can still prepare slides of the placenta. These slides can be reviewed at a later date by a placental pathologist.

There is concern that more lawsuits will be defended to avoid the settlement-reporting requirements of the National Data Bank. This could ultimately result in an increase in malpractice premiums.

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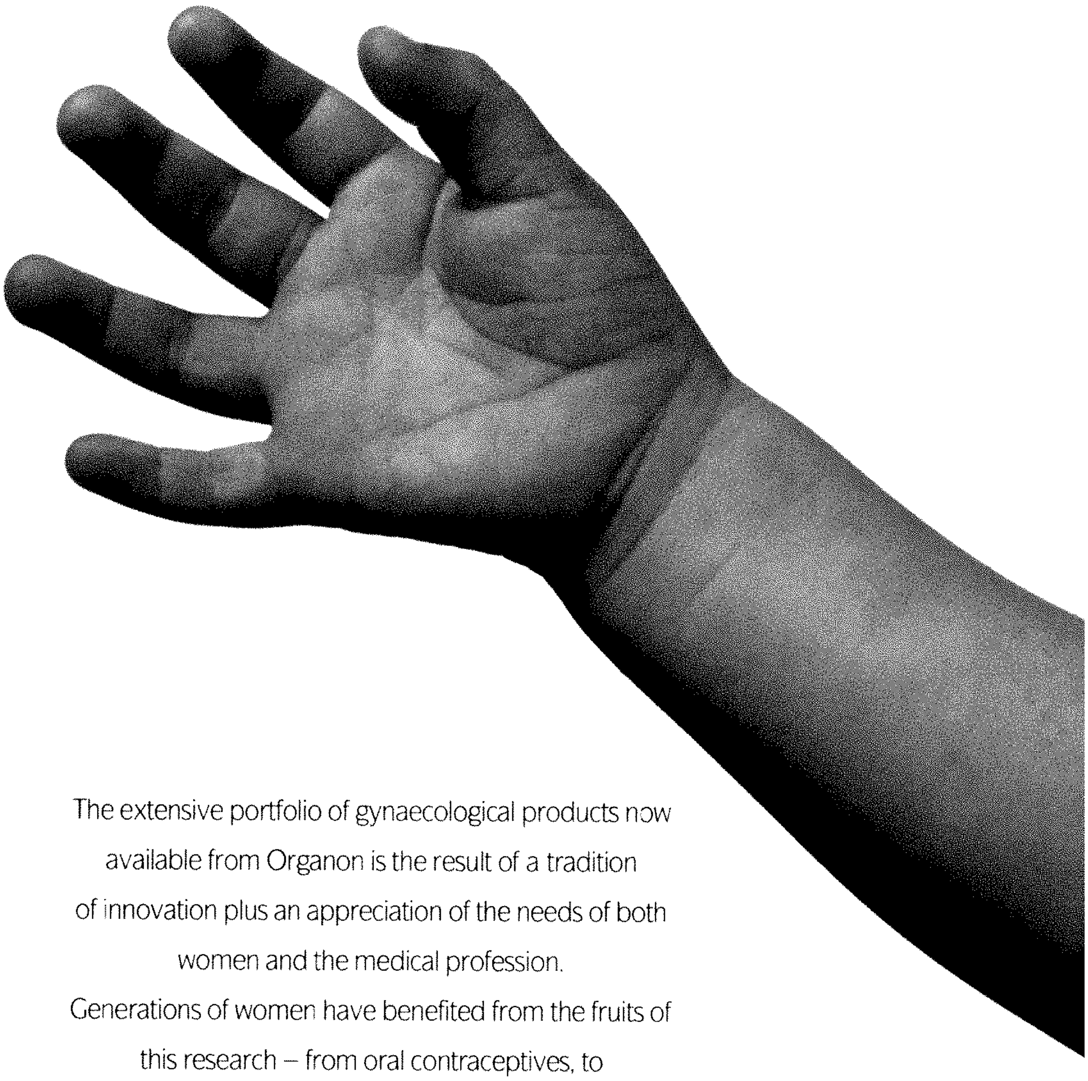

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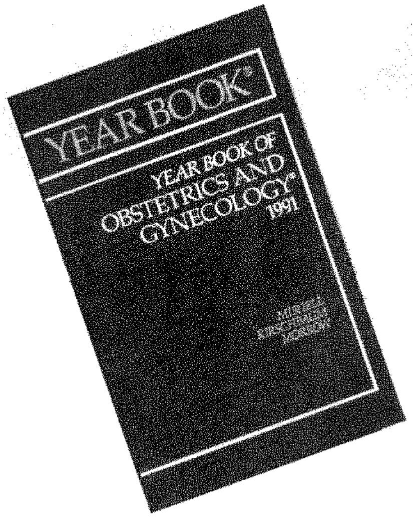

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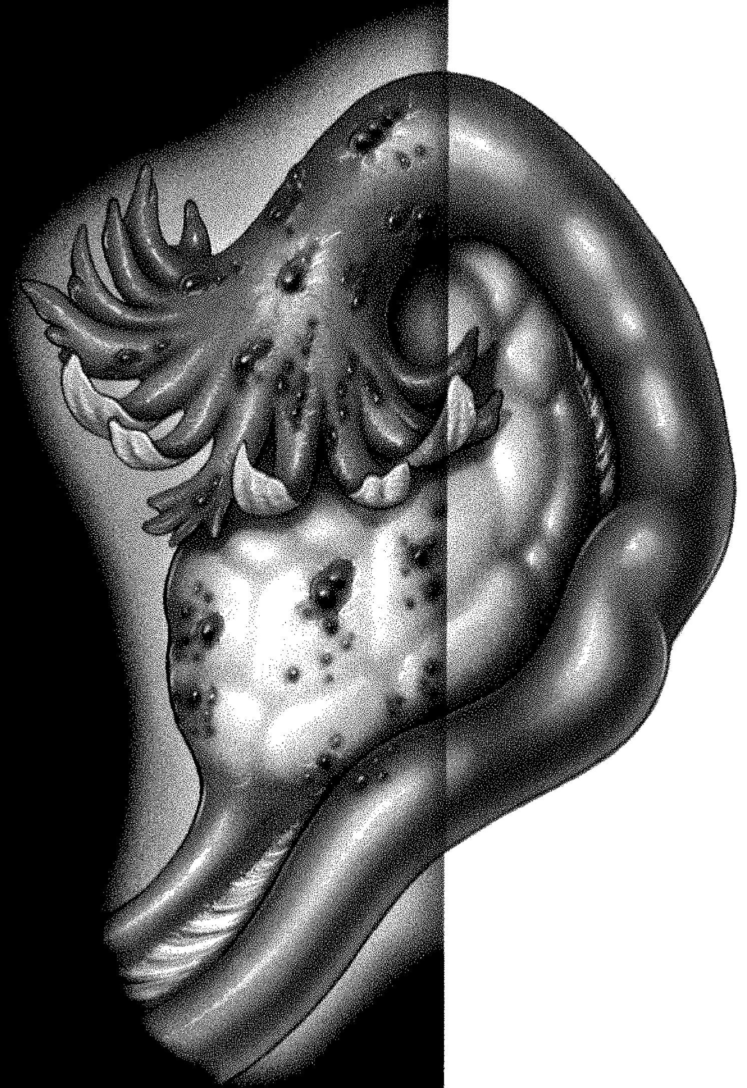

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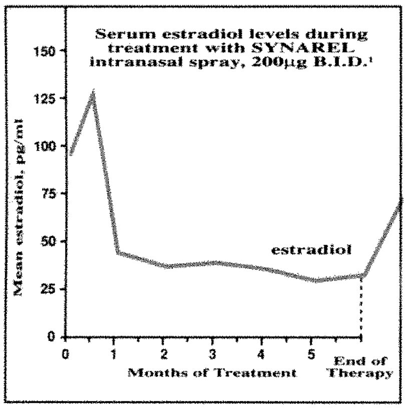

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Trabecular Bone of Vertebrae QCT* (mg/cc) n=23	161.3	147.6 −8.7%	152.0 −4.9%
Lumbar Vertebrae DPA† (g/cm²) n=6	0.98	0.93 −5.9%	0.96 −1.4%
Distal Radius SPA‡ (Units) n=6	40.2	39.5 −1.6%	39.6 −0.8%

*Quantitative computed tomography ‡Single photon absorptiometry
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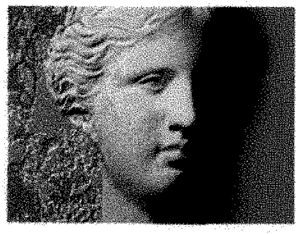
†Gonadotropin-releasing hormone analog.
[1] Syntex document #829-265-A.
[2] Syntex document #829-265-B.
Please see brief summary of prescribing information on next page.



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BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.
INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.
CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.
WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.
When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.
PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.
Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.
Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.
Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.
Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.
Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.
Pediatric Use: Safety and effectiveness in children have not been established.
ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.
Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.
Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.
Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.
Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (±SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.
Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.
OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

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Changes in lipids and lipoproteins with long-term estrogen deficiency and hormone replacement therapy

R. Don Gambrell, Jr., MD,[a] and Ana-Zully Teran, MD[b]
Augusta, Georgia

Cross-sectional data of the long-term effects of estrogens, androgens, and progestogens on lipids and lipoproteins were obtained in 556 postmenopausal women aged 24 to 85 years with follow-up for 1 to 44 years. Baseline values were obtained in 155 women from <1 year up to 30 years after menopause. Total cholesterol and low-density lipoprotein cholesterol tended to rise during the early postmenopausal years while high-density lipoprotein cholesterol did not change. Triglycerides were related to weight and were significantly different only between untreated women of normal weight (128.3 ± 7.80 mg/dl) and hormone users weighing >200 pounds (252.9 ± 9.44 mg/dl), $p \leq 0.001$. Although mean high-density lipoprotein cholesterol was lower with both C-21 progestogens (64.5 ± 4.16 mg/dl) and C-19 progestogens (61.9 ± 3.84 mg/dl), there were no statistically significant differences on comparison with levels in the unopposed estrogen users (67.0 ± 3.94 mg/dl). Smoking significantly depressed high-density lipoprotein cholesterol in both hormone users ($p \leq 0.001$) and untreated women ($p \leq 0.001$). Added progestogens do not adversely affect lipids and lipoproteins over the long term when adequate dosages of estrogens are used. (AM J OBSTET GYNECOL 1991;165:307-17.)

Key words: Progestogens, androgens, estrogens, lipids, lipoproteins

One of the greatest benefits that will accrue to postmenopausal estrogen users will be the prevention of cardiovascular disease.[1-9] With most types, dosages, and routes of administration, serum lipid patterns improve, primarily by a reduction in total cholesterol and low-density lipoprotein (LDL) cholesterol with an increase in high-density lipoprotein (HDL) cholesterol, thus improving the LDL/HDL ratio. Concern has been expressed that added progestogens to estrogen replacement therapy regimens may adversely affect the estrogen-induced beneficial effects on lipids, thus negating the benefit of hormone replacement on heart disease. Most of this is based on short-term studies of estrogen-progestogen use and speculation from old oral contraceptive data that postmenopausal hormone therapy will have similar effects.[10, 11] Although HDL cholesterol levels do not change with estrogen deficiency, LDL cholesterol and total cholesterol may increase. Estrogen therapy may increase HDL cholesterol while decreasing total cholesterol, primarily by decreasing LDL cholesterol.[12]

What is most important is not the short-term effects since most women will receive hormone replacement for many years. Previously published from our institution was a prospective intermediate-term study of a standard dosage of estrogen and comparison of the effects of three different progestogens on lipoproteins over a 6- to 18-month period.[13] Patients have received hormone replacement therapy for >50 years at the Medical College of Georgia. Cross-sectional data of the really long-term effects of estrogens, androgens, and progestogens on lipids and lipoproteins constitute the basis for this report.

Material and methods

There were 556 postmenopausal women who had been seen within the previous 6 months so that current status was known. Their ages ranged from 24 to 85 years with a mean of 57.14 ± 10.56 years. (The 24-year-old patient had androgen insensitivity syndrome with gonads removed at age 2 years; she has received hormone replacement for 12 years.) Hormone replacement has been given to these women for 1 to 44 years with a mean duration of therapy of 11.97 ± 8.11 years. Unopposed estrogen and/or androgen therapy, mostly in the form of pellet implants, was given to 132 of these patients whereas 424 also received progestogens. Baseline values of lipids and lipoproteins before hormone replacement were taken from <1 year up to 30 years after menopause in 155 of these 556 patients. In addition to measuring total cholesterol and triglycerides, fractionation of the lipoproteins was performed by electrophoresis into HDL, very LDL (VLDL), and LDL cholesterol with an interassay coefficient of variation of

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From the Departments of Endocrinology,[a, b] Obstetrics and Gynecology,[a] and Physiology,[b] Medical College of Georgia.

Presented at the Fifty-third Annual Meeting of the South Atlantic Association of Obstetricians and Gynecologists, Hot Springs, Virginia, January 27-30, 1991.

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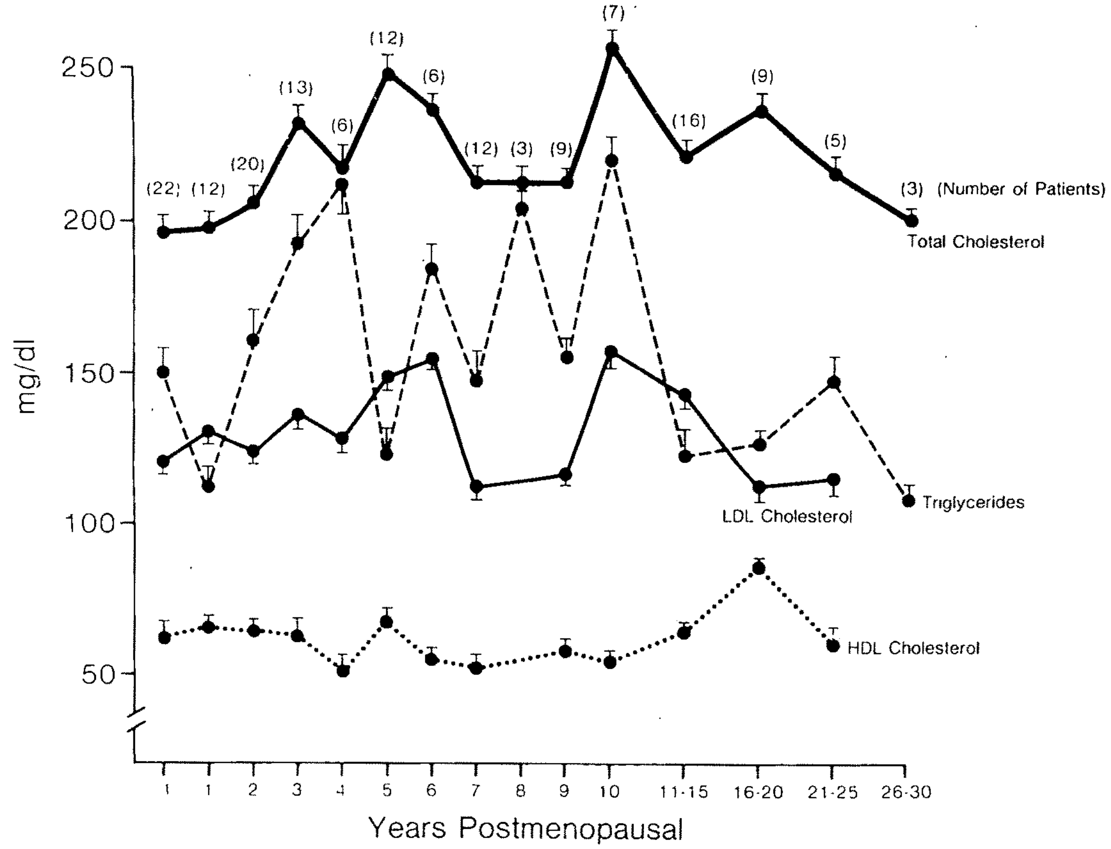

Fig. 1. Mean values of total cholesterol, triglycerides, LDL cholesterol, and HDL cholesterol at baseline before hormone replacement therapy in 155 women from <1 year up to 30 years after the last menstrual period. All values are expressed as mean ± SE. Number of patients at each year is shown in parentheses.

±3.5%. Hormone replacement therapy was given in various combinations but mostly with subcutaneous implantation of 25 mg estradiol pellets and 75 mg testosterone pellets every 4 to 6 months. The subgroups of hormone users were heterogeneous, but 494 women (88.8%) were treated with subcutaneous pellets whereas 404 patients (72.7%) received one or two pellets of estradiol and one or two pellets of testosterone. C-21 progestogens used included 2.5 to 10 mg medroxyprogesterone acetate, 25 to 100 mg progesterone suppositories, and 100 to 300 mg micronized progesterone daily by mouth for 10 to 13 days each month or menstrual cycle. C-19 progestogens used were 2.5 to 5 mg of norethindrone acetate, 2.5 to 5 mg of norethindrone, and 0.075 to 0.150 mg of norgestrel. Most patients were given cyclic progestogen therapy although a few used continuous progestogens. Oral estrogens, including 0.625 to 1.25 mg conjugated estrogens, 1 to 2 mg micronized estradiol, 1.25 to 2.5 mg estropipate, and 0.625 to 1.25 mg esterified estrogens, were given to a few patients. A few patients also used oral estrogen-androgen combinations, either 0.625 mg esterified estrogens and 1.25 mg methyltestosterone or 1.25 mg esterified estrogens and 2.5 mg methyltestosterone, and a few patients were treated with 0.05 to 0.1 mg transdermal estradiol. Statistical analysis of the data was performed by the two-way analysis of variance.

Results

Fig. 1 indicates the baseline values before hormone replacement in 155 of the 556 patients from <1 to 30 years after menopause. Values are expressed as mean + SEM. Total cholesterol tended to rise from <200 mg/dl in the early postmenopausal years to a mean of 248.2 ± 7.21 mg/dl at 5 years after the last menstrual period. LDL cholesterol also tended to increase from a mean of 120.3 ± 4.15 mg/dl in the first year after menopause to 155.0 ± 3.16 mg/dl at 6 years. Triglycerides were most variable, ranging from 114.2 ± 5.44 mg/dl at 1 year to 213.0 ± 10.34 mg/dl at 4 years. Triglycerides continued to fluctuate until more than 10 years after menopause when values ranged from 124.5 ± 6.97 to 148.4 ± 7.21 mg/dl. HDL cholesterol did not change appreciably with long-term estrogen deficiency. Mean values of HDL cholesterol ranged from 65 to 75 mg/dl throughout the early

Table I. Lipid profiles in women receiving unopposed estrogens and androgens

Hormone therapy	*No. of patients*	*Total cholesterol (mg/dl, mean ± SE)*	*Triglycerides (mg/dl, mean ± SE)*	*HDL cholesterol (mg/dl, mean ± SE)*	*LDL cholesterol (mg/dl, mean ± SE)*	*Total cholesterol/HDL ratio (mean ± SE)*	*LDL/HDL ratio (mean ± SE)*
50 mg Estradiol plus 75 mg testosterone	30	209.4 ± 5.53	157.7 ± 7.56	65.9 ± 4.94	123.0 ± 4.96	3.51 ± 1.05	2.10 ± 0.90
150 mg Testosterone plus 25 mg estradiol	24	227.4 ± 6.10	138.8 ± 7.10	59.6 ± 3.85	146.0 ± 6.73	4.09 ± 0.61	2.68 ± 1.12
150 mg Testosterone plus 50 mg estradiol	23	218.2 ± 5.58	173.7 ± 8.02	62.7 ± 3.51	133.5 ± 5.49	3.69 ± 0.84	2.24 ± 0.85
75 mg Estradiol plus 75 mg testosterone	14	205.4 ± 6.02	164.0 ± 8.86	74.9 ± 3.59	113.4 ± 5.61	2.84 ± 0.73	1.57 ± 0.66
25 or 50 mg Estradiol	6	228.8 ± 5.09	142.2 ± 6.87	75.0 ± 3.64	141.0 ± 4.13	3.47 ± 1.00	2.15 ± 0.89
25 mg Estradiol plus 75 mg testosterone	6	218.0 ± 3.21	172.8 ± 6.40	76.8 ± 3.08	117.2 ± 2.04	3.03 ± 0.62	1.67 ± 0.51
150 mg Testosterone	6	219.7 ± 5.08	189.5 ± 9.78	61.3 ± 3.12	135.7 ± 4.88	4.04 ± 1.06	2.57 ± 0.95
Low oral estrogen	9	211.7 ± 5.37	229.4 ± 10.92	70.5 ± 3.50	131.0 ± 4.65	3.45 ± 0.94	1.94 ± 0.76
High oral estrogen	14	242.1 ± 5.13	151.6 ± 7.38	73.3 ± 3.00	140.3 ± 5.35	3.34 ± 0.68	1.89 ± 0.62
Oral estrogen plus testosterone	0	—	—	—	—	—	—
TOTAL	132	219.1 ± 5.68	163.4 ± 7.70	67.0 ± 3.94	131.5 ± 5.66	3.55 ± 0.83	2.14 ± 0.91

postmenopausal years with a mean of 55.7 ± 1.70 mg/dl at 10 years after menopause.

Fig. 2 compares lipid and lipoprotein levels in the unopposed estrogen and/or androgen users with those of the estrogen-androgen-progestogen users during 1 to >36 years of hormone replacement. Total cholesterol levels ranged from 193.2 ± 5.42 to 213.3 ± 6.69 mg/dl during the first 5 years of hormone therapy including progestogens compared with a range of 193.0 ± 5.60 to 228.3 ± 4.55 mg/dl in the unopposed hormone users. After 20 years of hormone replacement, total cholesterol ranged from 191.3 ± 3.90 to 212.5 ± 5.68 mg/dl in hormone users including progestogens compared with 211.3 ± 6.97 to 233.6 ± 7.01 mg/dl in the unopposed hormone users. Overall, total cholesterol was >240 mg/dl in 21.9% of the unopposed hormone users and 11.8% of the estrogen-progestogen users. LDL cholesterol ranged from 109.4 ± 4.61 mg/dl at 2 years of estrogen-progestogen therapy to 129.3 ± 5.73 between 21 and 25 years. In the unopposed estrogen-androgen users, mean LDL cholesterol varied from 100.8 ± 3.48 mg/dl at 4 years of therapy to 164.7 ± 8.43 mg/dl between 26 and 30 years of treatment. Overall, 23.1% of the estrogen users had LDL levels >138 mg/dl as did 17.1% of the estrogen-progestogen users. Mean HDL cholesterol varied from 57.3 ± 2.99 to 69.4 ± 3.69 mg/dl with up to 36 years of estrogen-progestogen use. In the unopposed estrogen group mean HDL varied from 56.0 ± 2.88 to 73.8 ± 4.10 mg/dl over the long term. Where 78.6% of the estrogen users had HDL values >54 mg/ml, as did 69.7% of the estrogen-progestogen users, only 14.3% of the estrogen users and 17.1% of the estrogen-progestogen users had HDL values <50 mg/dl.

Lipid profiles in the unopposed estrogen-androgen users are given in Table I; they are listed by groups according to the combination of therapy. The highest mean cholesterol level in the pellet implant groups (228.8 ± 5.09 mg/dl) was observed in those women receiving 50 to 75 mg estradiol. This group also had a high mean HDL value (75.0 ± 3.64 mg/dl) so that the total cholesterol/HDL ratio was 3.47 ± 1.00, which was below average risk for heart disease (4.44 mg/dl) for our laboratory. In fact, the mean total cholesterol/HDL ratios were below average risk for heart disease in all groups, and only 7.7% of the unopposed estrogen users and 7.9% of the estrogen-progestogen users had total cholesterol/HDL ratios that were above average risk for heart disease. The highest mean cholesterol value (242.1 ± 5.13 mg/dl) was in the high-dosage oral estrogen users (1.25 mg conjugated estrogens and 2 mg micronized estradiol or 2.5 mg estropipate); however, these women also had an HDL level of 73.3 ± 3.00 mg/dl, so their total cholesterol/HDL ratio was below average risk at 3.34 ± 0.68. Even though the LDL cholesterol mean was elevated above normal (86 to 138 mg/dl) in the patients receiving 150 mg testosterone

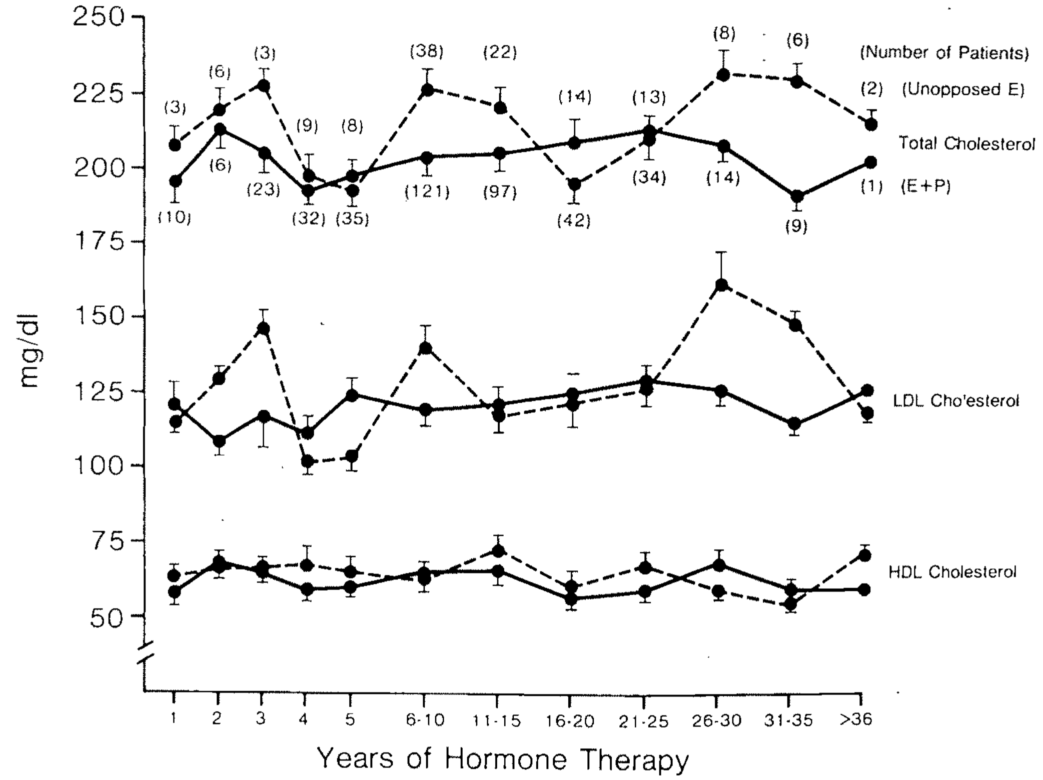

Fig. 2. Mean values of total cholesterol, LDL cholesterol, and HDL cholesterol compared in unopposed estrogen users *(Unopposed E)* and estrogen-progestogen users *(E + P)* at 1 to 44 years of hormone replacement therapy. All values are expressed as mean ± SE. Number of patients at each year is shown in parentheses. Dotted lines with *numbers above graph* represent unopposed estrogen users and *solid lines* with *numbers below graph* represent estrogen-progestogen users.

and 25 mg estradiol at 146.0 ± 6.73 mg/dl, LDL/HDL ratios in these women were below average risk for heart disease (3.22) at 2.68 ± 1.12. In fact the LDL/HDL ratio was below average risk for all groups of unopposed estrogen-androgen users.

The lipid profiles for women receiving estrogens, androgens, and C-21 progestogens are listed in Table II. Mean total cholesterol levels for all groups were close to or below 200 mg/dl, which is ideal. Mean triglyceride levels were above normal (30 to 150 mg/dl) in two groups, those receiving 150 mg testosterone and 25 mg estradiol and those receiving 75 mg estradiol and 75 mg testosterone. Mean HDL cholesterol values for all groups were in the average-risk (54 to 59 mg/dl) to low-risk (59 to 74 mg/dl) range for cardiovascular disease. In both of the oral estrogen–C-21 progestogen groups, mean HDL cholesterol levels were in the very-low-risk range (>74 mg/dl) for heart disease. Mean LDL cholesterol values were well within the normal range (86 to 138 mg/dl). The total cholesterol/HDL ratios were all well below the 4.44 ratio for average risk. In fact, several groups were below the 1/2 average risk ratio (3.27). LDL/HDL ratios all were below the average risk ratio (3.22), some approaching the 1/2 average risk ratio (1.47).

Lipid profiles for women being treated with hormone replacement including C-19 progestogens are shown in Table III. All of the mean total cholesterol values were near the ideal range of ≤200 mg/dl. Triglycerides were elevated in the groups receiving a predominance of estrogen: 75 mg estradiol and 75 mg testosterone, 50 to 75 mg estradiol, and low-dosage oral estrogen. Mean HDL levels were within the average-risk range except in the two patients being treated with 75 mg testosterone, 25 mg estradiol, and C-19 progestogens. The HDL levels were 44 and 48 mg/dl, but with both having total cholesterol levels <200, the total cholesterol/HDL ratios were 4.44 and 3.79, respectively. Mean LDL cholesterol levels were normal for all groups, including the one receiving 150 mg testosterone and C-19 progestogen. Mean total cholesterol/HDL ratios and LDL/HDL ratios were below average risk for cardiovascular disease in all groups. Overall, total cholesterol/HDL ratios were above average risk for cardiovascular disease in 7.7% of the unopposed estrogen users and 7.9% of the estrogen-progestogen users. LDL/HDL ratios were above average risk in 7.1% of the unopposed estrogen users and 3.9% of the progestogen users.

In comparing HDL cholesterol values in the unop-

Table II. Lipid profiles in women using estrogens, androgens and C-21 progestogens

Hormone therapy	*No. of patients*	*Total cholesterol (mg/dl, mean ± SE)*	*Triglycerides (mg/dl, mean ± SE)*	*HDL cholesterol (mg/dl, mean ± SE)*	*LDL cholesterol (mg/dl, mean ± SE)*	*Total cholesterol/HDL ratio (mean ± SE)*	*LDL/HDL ratio (mean ± SE)*
50 mg Estradiol plus 75 mg testosterone	42	200.2 ± 6.32*	133.8 ± 7.40	66.5 ± 4.28	119.9 ± 5.40	3.22 ± 0.86	2.04 ± 0.87
150 mg Testosterone plus 25 mg estradiol	75	207.2 ± 5.64	158.1 ± 9.95	59.8 ± 3.91	127.4 ± 5.32	3.88 ± 1.08	2.39 ± 0.95
150 mg Testosterone plus 50 mg estradiol	59	198.7 ± 6.09	145.6 ± 9.88	63.3 ± 4.1	117.2 ± 5.24	3.43 ± 1.06	2.04 ± 0.82
75 mg Estradiol plus 75 mg testosterone	10	196.6 ± 6.16	189.0 ± 8.57	61.3 ± 3.37	106.8 ± 5.59	3.27 ± 0.77	1.94 ± 0.70
25 or 50 mg Estradiol	19	203.8 ± 5.29	129.8 ± 8.49	65.3 ± 4.13	119.8 ± 5.16	3.31 ± 0.94	1.59 ± 0.82
25 mg Estradiol plus 75 mg testosterone	0	—	—	—	—	—	—
150 mg Testosterone	3	184.7 ± 2.65	126.3 ± 2.24	77.0 ± 4.58	120.0 ± 5.26	3.21 ± 0.96	1.90 ± 0.92
Low oral estrogen	14	221.4 ± 6.73	121.8 ± 5.94	83.4 ± 3.70	125.9 ± 5.88	3.20 ± 0.92	1.87 ± 0.83
High oral estrogen	7	213.7 ± 5.42	146.1 ± 7.44	79.2 ± 4.07	116.8 ± 4.87	2.75 ± 0.63	1.53 ± 0.62
Oral estrogen plus testosterone	12	208.8 ± 5.38	100.8 ± 5.32	61.2 ± 3.78	130.4 ± 5.35	3.75 ± 0.99	2.35 ± 0.85
TOTAL	241	204.0 ± 5.87	144.1 ± 8.68	64.5 ± 4.16	121.8 ± 5.34	3.50 ± 1.01	2.10 ± 0.88

posed estrogen-androgen users with those in women who also received progestogens, mean values were higher in the unopposed estrogen users (67.0 ± 3.94 mg/dl) than in those with added C-21 progestogens (64.5 ± 4.16 mg/dl) or C-19 progestogens (61.9 ± 3.84 mg/dl) (Tables I, II, and III). These mean values were above the average-risk range (54 to 59 mg/dl) into the low-risk range (60 to 74 mg/dl). A two-way analysis of variance did not reveal any statistically significant difference among the three groups.

Of the many other variables relating to both lipids and heart disease, including diet, activity, family history, and smoking, the relationship to weight is illustrated in Fig. 3. Baseline values before hormone replacement are compared with values after hormone use according to the patient's weight. There were no differences in total cholesterol related to weight in the untreated women. Total cholesterol was 218.4 ± 6.45 mg/dl at baseline in those with normal weight, 217.4 ± 6.40 mg/dl in those weighing between 150 and 200 pounds, and 217.6 ± 6.59 mg/dl in those weighing >200 pounds. There was a moderate increase corresponding to increased weight in the hormone users: 202.8 ± 3.35 mg/dl with normal weight, 213.2 ± 4.16 mg/dl in those weighing between 150 and 200 pounds, and 231.3 ± 6.33 mg/dl in those weighing >200 pounds. Triglycerides increased markedly with increased weight both at baseline and with hormone use. At baseline, women weighing <150 pounds had mean triglyceride levels of 128.3 ± 7.80 mg/dl, compared with 147.6 ± 7.88 mg/dl in those weighing between 150 and 200 pounds and 234.4 ± 9.71 mg/dl in those weighing >200 pounds. In the hormone users triglycerides also increased markedly with increased weight: 132.7 ± 3.91 mg/dl in those with normal weight, 169.6 ± 6.45 mg/dl when weight was between 150 and 200 pounds, and 252.9 ± 9.44 mg/dl in those weighing >200 pounds. However, the only statistically significant difference was between the untreated women of normal weight (128.3 ± 7.80 mg/dl) and the hormone users weighing >200 pounds (252.9 ± 9.44 mg/dl), $p \leq 0.001$. HDL cholesterol decreased with increasing weight at baseline before hormone replacement from a mean of 64.9 ± 4.42 mg/dl with normal weight to 59.4 ± 4.13 mg/dl in those between 150 and 200 pounds and to 44.0 ± 3.16 mg/dl in those weighing >200 pounds. HDL cholesterol likewise decreased with increasing weight in the hormone users from 65.7 ± 2.50 mg/dl at normal weight to 59.9 ± 2.63 mg/dl between 150 and 200 pounds and to 54.3 ± 3.41 mg/dl in those weighing >200 pounds. There were similar changes as a result of increasing obesity with both LDL cholesterol and the total cholesterol/HDL ratio in that both increased with increasing weight at baseline and with hormone use.

The relationship of smoking and hormone use on lipid patterns are shown in Fig. 4. In every instance, except for HDL cholesterol, the hormone users, both nonsmoking and smoking, had lower mean values than the baseline before hormone replacement was begun.

Table III. Lipid profiles in women receiving estrogens, androgens, and carbon 19 progestogens

Hormone therapy	*No. of patients*	*Total cholesterol (mg/dl, mean ± SE)*	*Triglycerides (mg/dl, mean ± SE)*	*HDL cholesterol (mg/dl, mean ± SE)*	*LDL cholesterol (mg/dl, mean ± SE)*	*Total cholesterol/HDL ratio (mean ± SE)*	*LDL/HDL ratio (mean ± SE)*
50 mg Estradiol plus 75 mg testosterone	29	198.5 ± 5.69*	134.6 ± 8.87	65.9 ± 3.67	116.6 ± 5.12	3.24 ± 0.87	1.95 ± 0.76
150 mg Testosterone plus 25 mg Estradiol	65	199.3 ± 5.59	134.2 ± 8.30	61.3 ± 4.18	117.9 ± 5.49	3.45 ± 1.01	2.11 ± 0.92
150 mg Testosterone plus 50 mg estradiol	47	209.0 ± 6.42	135.4 ± 7.63	62.1 ± 3.60	129.1 ± 6.26	3.56 ± 0.99	2.23 ± 0.93
75 mg Estradiol plus 75 mg testosterone	11	210.8 ± 5.38	199.6 ± 8.47	55.6 ± 4.10	125.6 ± 4.64	4.32 ± 0.99	2.56 ± 0.85
25 or 50 mg Estradiol	17	185.9 ± 5.41	161.3 ± 10.09	67.9 ± 3.34	100.3 ± 4.73	2.84 ± 0.84	1.56 ± 0.71
25 mg Estradiol plus 75 mg Testosterone	2	187.0 ± 2.24	151.0 ± 2.24	46.0 ± 1.41	117.0 ± 2.24	4.09 ± 0.56	2.55 ± 0.47
150 mg Testosterone	6	213.3 ± 4.70	133.8 ± 6.64	54.3 ± 2.86	141.3 ± 3.90	4.10 ± 0.67	2.77 ± 0.61
Low oral estrogen	2	208.5 ± 6.04	173.5 ± 4.06	65.5 ± 2.74	93.5 ± 2.55	3.36 ± 0.50	1.45 ± 0.51
High oral estrogen	0	—	—	—	—	—	—
Oral estrogen or testosterone	4	184.3 ± 5.62	102.3 ± 5.63	51.6 ± 1.97	124.3 ± 1.91	3.84 ± 0.85	2.59 ± 0.77
TOTAL	183	201.2 ± 5.73	140.9 ± 8.17	61.9 ± 3.84	120.0 ± 5.31	3.48 ± 0.96	2.12 ± 0.87

The mean HDL level in the smoking hormone users (56.3 ± 4.26 mg/dl) was between the mean HDL of nonsmoking untreated women (59.6 ± 4.04 mg/dl) and that of smoking untreated women (48.8 ± 3.69 mg/dl). The highest mean HDL cholesterol (63.5 ± 4.17 mg/dl) was observed in the nonsmoking hormone users. The only statistically significant differences in these groups were in HDL cholesterol values between the nonsmoking hormone users (63.5 ± 4.17 mg/dl) and the smoking hormone users (56.3 ± 4.26 mg/dl), $p \leq 0.001$, and between the nonsmoking hormone users and the smoking untreated women (48.8 ± 3.69 mg/dl), $p \leq 0.001$.

Four of these 556 postmenopausal women had evidence of coronary artery disease during hormone replacement therapy. Their clinical characteristics, years of hormone replacement at the time of cardiovascular event, and lipid values are given in Table IV. Although none has died of heart disease, two (patients 1 and 3) had cardiac catheterization because of angina and were treated with angioplasty and two (patients 2 and 4) underwent coronary bypass surgery after myocardial infarction. All were moderately obese but none were smokers. Although patient 1 had a total cholesterol level of 226 mg/dl, which was above the 200 mg/dl considered to be ideal, the rest of her lipid values were within the normal range. Triglycerides were elevated in three (patients 2, 3, and 4) and HDL cholesterol was quite low at 37 mg/dl in patient 3. Patient 4 had deteriorating lipid changes. At age 65, after 12 years of hormone replacement therapy, breast cancer was diagnosed; therefore the estrogen was discontinued and two pellets of testosterone (150 mg) were implanted every 6 months, along with C-19 progestogen given orally for 13 days every month. At diagnosis of the breast cancer her lipid pattern was elevated, except for HDL cholesterol. During the next 3 years, without the estrogen therapy, total cholesterol, triglycerides, LDL cholesterol, and total cholesterol/HDL ratio all increased while HDL decreased to 39 mg/dl in spite of a cholesterol-control diet. At age 68, after 3 years without estrogen replacement, a myocardial infarction occurred and she was treated with coronary bypass surgery. At 2 years after the myocardial infarction, she has lost some weight, is more carefully controlling cholesterol intake, and has continued to receive the androgen-progestogen replacement without estrogen. Lipid patterns are better but still compromised.

Comment

It is clear that there is a protective effect from cardiovascular disease with estrogen replacement therapy. How this is mediated is still to be firmly established. There is a role through lipids in that estrogens decrease total cholesterol, primarily through a decrease in LDL cholesterol. The total cholesterol/HDL cholesterol ratio is improved since estrogens also may increase HDL cholesterol. This may vary by type and dosage of es-

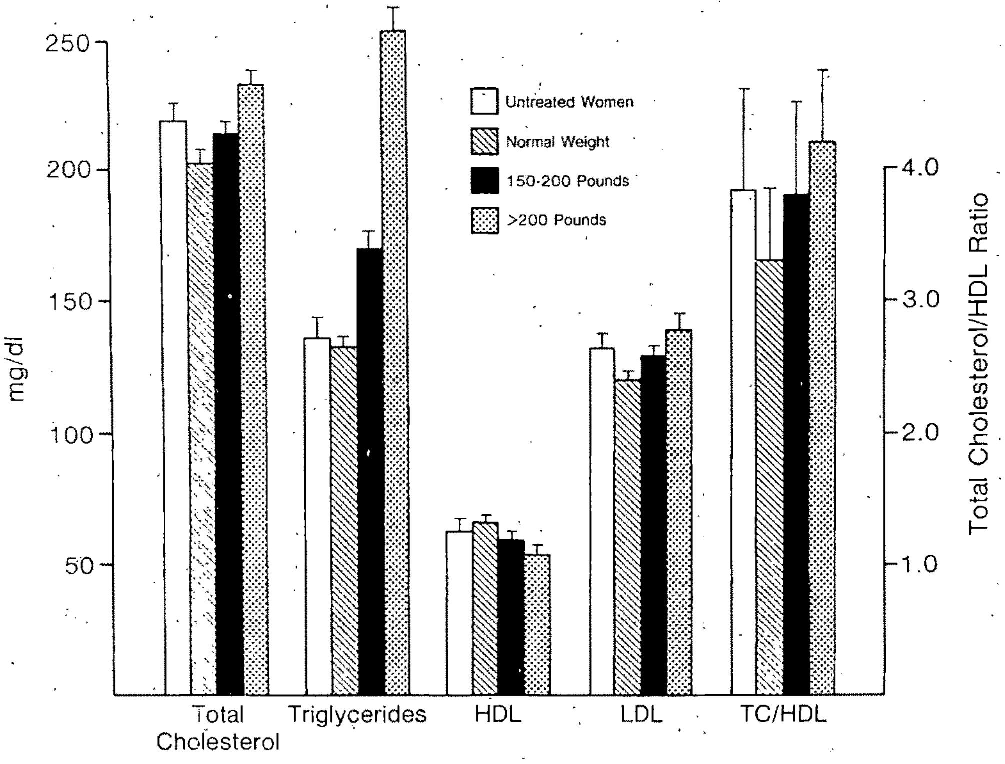

Fig. 3. Mean values of various lipids at baseline before hormone replacement *(Untreated women)* compared with values in hormone users according to weight. All values are expressed as mean ± SE.

trogen, route of administration, and particularly the duration of therapy. Nonoral routes of administration of estrogen replacement, such as the subcutaneous estradiol pellets used in this study, have been shown to significantly increase HDL cholesterol.[14] In a comparison of estradiol administered by subcutaneous pellet or transdermal patch, HDL cholesterol increased significantly by 12 weeks with the pellet but 24 weeks was required to show a significant increase with the patch. The total cholesterol/HDL ratio also was significantly decreased at 12 weeks with the estradiol pellet whereas 24 weeks was necessary to show a significant decrease with the estradiol patch.

A few studies have shown that there may be an adverse effect of progestogens, particularly on HDL cholesterol, and primarily by the 19-nortestosterone progestogens.[10, 15] However, most of these are short-term studies, usually of 3 months' duration. What is really important are the long-term effects of hormone replacement because the benefits of hormone replacement, such as prevention of osteoporosis and cardiovascular disease, require long-term use. Our data would indicate that there are really no adverse effects of added progestogens over the long term since the majority of the various lipids and lipoproteins remain within the normal range. Even when androgens in the form of testosterone pellets are included in hormone replacement, there was little difference in mean HDL cholesterol values: 67.0 ± 3.94 mg/dl in the unopposed estrogen-androgen users, 64.5 ± 4.16 mg/dl in the estrogen–androgen–C-21 progestogen users, and 61.9 ± 3.84 mg/dl in the estrogen–androgen–C-19 progestogen users. The total cholesterol/HDL ratios were likewise very favorable, 3.55 ± 0.83, 3.50 ± 1.01, and 3.48 ± 0.96, respectively, all well below the 4.44 average-risk ratio. Other long-term studies indicate a significantly improved LDL/HDL ratio with estrogens combined with C-19 progestogens[16, 17] or estrogens combined with C-21 progestogens.[18]

It may be the dosage of estrogen that is most important in improving the lipid pattern to decrease the risk for cardiovascular disease. In a study comparing different dosages of micronized estradiol, 1, 2, and 4 mg, combined with a standard dosage of a C-19 progestogen, norethindrone acetate 1 mg for 11 days, HDL cholesterol increased significantly only with 2 and 4 mg of estradiol.[19] Total cholesterol decreased significantly only with 4 mg estradiol combined with 1 mg norethindrone acetate, but there were significant decreases in LDL cholesterol with all three dosages of estradiol. In studies from the Lipid Research Clinics there was also a dosage response between 0.625 and 1.25 mg conjugated estrogens.[12] Total cholesterol increased slightly with 0.625 mg while it decreased with 1.25 mg. HDL

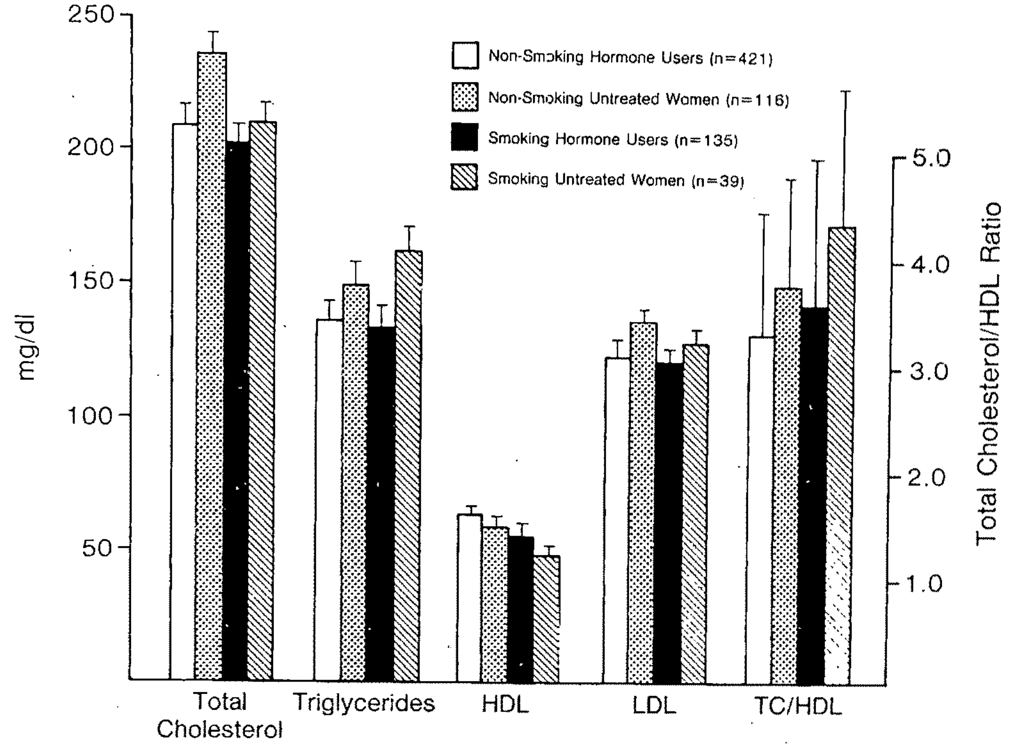

Fig. 4. Mean values of various lipids at baseline before hormone replacement *(Untreated women)* compared with values in hormone users according to smoking status. All values are expressed as mean ± SE. Numbers in each group are shown in parentheses.

Table IV. Patients with coronary artery disease

Patient No.	Age (yr)	Weight (lb)	Duration of hormone therapy (yr)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	Total cholesterol/HDL ratio
1	69	189	8	226	97	74	127	3.03
2	66	174	15	223	555	62	145	3.58
3	54	165	12	226	303	37	120	6.11
4	65	189	—	254	228	56	157	4.54
—	—	189	—	256	269	46	169	5.54
—	—	188	—	241	260	49	144	4.94
—	—	194	—	275	245	47	204	5.85
—	68	191	15	269	264	39	203	6.83

cholesterol increased and LDL decreased with both dosages of conjugated estrogens, but the change was greater with 1.25 mg. Our data, as well as these other long-term studies, indicate that progestogens, even androgenic progestogens, do not negate the estrogen-induced increase in HDL cholesterol and decrease in LDL cholesterol over the long term, especially when adequate dosages of estrogens are given.

Factors that seem to affect lipids and lipoproteins more than progestogens in estrogen users are obesity and smoking. Increasing weight gave higher mean values of total cholesterol and LDL cholesterol, and particularly triglycerides while decreasing the levels of HDL cholesterol. Smoking not only depressed HDL cholesterol but also increased total cholesterol, triglycerides, and LDL cholesterol in untreated women. According to our data in the 155 women who had baseline values measured before hormone replacement for up to 30 years after menopause, total cholesterol tends to rise within the first few years of estrogen deficiency, primarily because of an increase in LDL cholesterol. Triglycerides also increase with estrogen deprivation but were very variable, more related to obesity. HDL cholesterol did not change after menopause, even with >25 years of estrogen deficiency. A recent study from Denmark also observed significant increases in total cholesterol, LDL cholesterol, and triglycerides after menopause.[20] They also observed a significant decrease in HDL cholesterol but the decline occurred gradually over the 2 years preceding the cessation of menses with no further decrease after menopause.

That the tremendous cardioprotective effect of estrogen replacement is not totally mediated through lipids seems to be indicated by the individual lipid patterns in four of our 556 postmenopausal women with coronary artery disease. All were obese but none were smokers. The lipid pattern was entirely normal in one, triglycerides were elevated in three, but only one had a depressed HDL cholesterol level. One patient who had a deteriorating lipid pattern during the 3 years after estrogen therapy was discontinued from the study because of breast cancer, which probably played a major role in the subsequent myocardial infarction. However, other factors may be important, such as a direct effect of estrogen on arterial walls because estrogen receptors have been identified. Estrogens may improve vascular blood flow, even dilate coronary arteries, decrease platelet adhesiveness, and also be mediated through prostacyclin and thromboxane metabolism.[21, 22]

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Editors' note: This manuscript was revised after these discussions were received.

Discussion

DR. WALLACE C. NUNLEY, JR., Charlotte, North Carolina. Cardiovascular disease is the leading cause of morbidity and death among postmenopausal women in the United States. Previous epidemiologic studies have determined that the incidence of cardiovascular disease for women begins to increase before and "accelerates" after menopause. The loss of natural estrogen production at the time of menopause seems to have an adverse effect on the female cardiovascular system.

Attention has been directed to an individual's lipid profile as a predictor for cardiovascular disease. The higher the total cholesterol, the higher the LDL, and the lower the HDL levels herald an increased risk of disease. With the onset of menopause, there are significant increases in the levels of total cholesterol and LDL, as well as triglycerides, compared with the premenopausal state. The level of HDL cholesterol begins to decline gradually, approximately 2 years before menopause.[1] These alterations occur without changes in the individual's weight and are thereby attributed mainly to the loss of estrogen production. Thus the stage is set for the hypothesis that estrogen replacement may alter one's lipid levels to a more favorable profile and decrease cardiovascular disease risk in the menopausal woman, a practice of true preventive medicine. Bush et al.,[2] in the Lipid Research Clinics Program, published data in 1987 that concluded that, after a mean of 8.5 years of estrogen replacement therapy, women not only had a lower incidence of cardiovascular deaths than non–estrogen users had but also had a significantly lower risk of death from all causes. The beneficial lipid effects of estrogen replacement therapy are dependent on dosage, duration of treatment, and delivery system.

Because of consistently documented increases in the risk of adenocarcinoma of the endometrium with estrogen replacement therapy, the addition of a progestin was proposed. Combination hormone replacement therapy eliminated the endometrial adenocarcinoma risk.[3] However, progestin usage created another health concern, namely, potential adverse effect on lipid profile.

Before the current report of Drs. Gambrell and Teran, information was available that estrogen-progestin therapy, in various dosages and with different compounds, could be used without significantly adversely affecting the beneficial lipid alterations achieved with estrogen replacement alone. In the majority of reports, however, treatment was limited to a few weeks up to a maximum of 2 years.

The study presented today attempts to reassure clinicians who advocate this treatment in the postmenopausal woman. After a mean of 12 years of therapy, three groups of women (those treated with estrogens alone, estrogens plus C-21 progestins, and estrogens plus C-19 progestins) continued to have reassuring lipid ratio profiles. In addition, correlation of these lipid levels and ratios was applied to the incidence of cardiovascular disease. Only 4 of the 556 women had evidence of coronary artery disease during hormone replacement therapy, and all 4 were defined as obese, another risk factor. Although it is unclear as to which of the three groups these women belonged, the small absolute number affected tends to reassure clinicians that combined hormone replacement therapy in the doses described is not associated with increased risk of cardiovascular disease. Furthermore, as stated by the authors, the cardioprotective effect of estrogen replacement is not totally mediated through lipids.

Smoking increases one's risk of cardiovascular disease. In this presentation the HDL cholesterol level statistically was greater in the nonsmoking hormone users than in the smoking hormone users and the smoking untreated women. Smoking may accelerate estrogen metabolism, and thus smokers have lower endogenous estrogen levels than do nonsmokers. This may account for the changes depicted in the HDL cholesterol level. Quantity of cigarettes smoked and/or number of pack years were not defined in this study.

A total of 103 of the 132 (78%) unopposed estrogen users and 363 of the 424 (85.6%) estrogen-progestin users received concomitant androgen therapy. What effect, if any, did the addition of testosterone have on the lipid profile, because androgens can decrease HDL and increase LDL concentrations in certain clinical situations? Sherwin et al.[4] reported in an earlier study that the addition of 150 mg testosterone intramuscularly monthly did not yield different lipid levels compared with those of women receiving estrogen alone. Similarly, in this study the addition of androgens did not seem to affect adversely the individuals depicted.

According to Stanczyk et al.,[5] the use of a 25 mg estradiol pellet yields a peripheral estradiol level of 40 to 70 pg/ml over a 6-month period and two 25 mg pellets yield a level of 95 to 126 pg/ml over the same period of time. Higher estrogen levels should yield improved lipid levels and lipid ratio profiles. The study by Gambrell and Teran is consistent with this because their results yield a trend of improved lipid profiles with an increase in the number of estrogen pellets used.

Although not a randomized prospective study, this cross-sectional study has sufficient numbers of women at extended periods of time from menopause and treatment to add reassurance regarding risk of cardiovascular disease to clinicians and patients who use combined hormone replacement therapy.

I have the following questions for Dr. Gambrell:

1. With the knowledge gained from this study regarding the impact of long-term estrogen-progestin therapy on an individual's lipoprotein cholesterol profile, what is his current recommendation regarding combination therapy in a woman who has undergone hysterectomy?
2. In addition to the information gained today regarding long-term estrogen and/or androgen-progestin therapy on lipid profiles and incidence of cardiovascular disease, does Dr. Gambrell have any additional information regarding number of women experiencing wrist, hip, or spine fractures, or strokes, or breast cancer from this same cohort?
3. Did any women have peripheral estrogen levels measured in this cross-sectional study? Were any androgen levels measured to see if the addition of this hormone had any effect on the circulating estrogen level?

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Dr. Gambrell (Closing). For 6 years we have recommended that all women on a regimen of estrogen replacement, whether they do or do not have an intact uterus, be given a progestogen. Many physicians were doing this several years ago, and then the lipid issue arose and some backed off. There are two benefits from the added progestogen other than the prevention of endometrial cancer. There is added protection of the bones in that adding progestogen to estrogen replacement over a 10- to 12-year period can increase bone density. That is the only form of therapy known to do it. Our data, now confirmed by two others, show that the addition of a progestogen decreases the incidence of breast cancer for some women. This is controversial, as you well know, but there are more data coming out of New York later this year that will confirm this finding.

Do we have any fractures in our patients? We had no wrist or hip fractures. We don't know of any spine fractures, but there may be silent lumbar compression fractures. Unless roentgenography is being done for back pain, you may not pick these up. There were no strokes. Breast cancer developed in one patient after 12 years. We have had eight other patients on regimens of testosterone and progestogen only because they had breast cancer.

What about estrogen and androgen levels? With two pellets of 50 mg estradiol over the long term blood levels will be approximately 200 pg of estradiol. With two testosterone pellets the total testosterone level will range between 110 and 190 ng, which is higher than in most women. Free testosterone is usually 3 to 7 ng, which is the upper limit of normal for most women. When you are giving estrogen, bound testosterone levels are higher, so there aren't as many side effects from the androgens as you might expect.

Endometrial morphology in asymptomatic postmenopausal women

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Few data are available regarding endometrial histologic features in asymptomatic perimenopausal and postmenopausal women. This study encompasses endometrial biopsy specimens obtained from 801 such women before enrollment in a multicenter study of estrogen-progestin replacement. One endometrial cancer was found (0.13%); four additional biopsy specimens showed atypia (total 0.63%). The endometrium was atrophic in 373 (46.9%), proliferative in 133 (16.7%), secretory in 54 (6.8%), and hyperplastic in 41 (5.2%). Insufficient tissue for diagnosis was obtained in 195 (24.5%). We conclude that the yield for neoplasia is so low that screening endometrial biopsy is not justified in asymptomatic perimenopausal and postmenopausal women. (AM J OBSTET GYNECOL 1991;165:317-22.)

Key words: Histology, endometrial; menopause; carcinoma; endometrium; hyperplasia

Endometrial cancer occurs at an incidence rate of 0.72 per 1000 women.[1] In 1986 it was estimated that there were 36,000 new cases of endometrial cancer in the United States, compared with 14,000 cases of cervical cancer.[2]

Endometrial biopsy has been associated with a high degree of sensitivity and specificity for the detection of endometrial cancer in a patient population with symptoms.[3-5] The value of screening a postmenopausal population without symptoms is debatable. Recently, the Canadian Task Force on the Periodic Health Examination concluded that screening for endometrial cancer be excluded in postmenopausal women without symptoms.[6] They base their reasoning on the following facts. Endometrial cancer manifests early with vaginal bleeding and has a high 5-year survival rate (75%). No con-

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A complete list of the principal investigators who participated in this study is shown at the end of this article.

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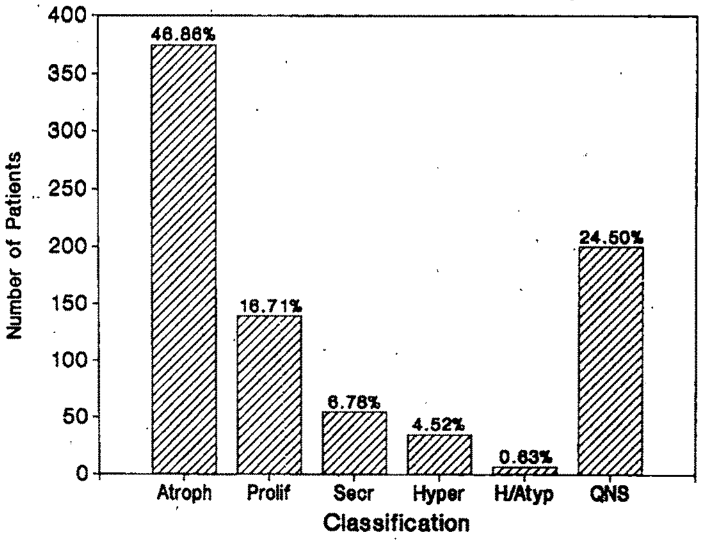

Fig. 1. Bar graph indentifying number and percent of endometrial histologic findings in asymptomatic perimenopausal and postmenopausal women. *Atroph*, Atrophic; *Prolif*, proliferative; *Secr*, secretory; *Hyper*, hyperplasia (simple, cystic, and adenomatous); *H/Atyp*, atypical cellular findings; *QNS*, insufficient tissue for diagnosis.

trol trials have been conducted to evaluate the effectiveness of prevention or treatment in changing the outcome of endometrial cancer. Last, screening of high-risk women would detect only 50% of cases of endometrial cancer.

Several reported techniques can be used for obtaining both cytologic and histologic material from the uterine endometrial cavity in outpatients.[7,8] On the basis of results from a large screening of women without symptoms ($N = 2586$) aged >45, Koss et al.[9] have recommended routine screening of women for endometrial carcinoma because they found an incidence of 6.96 cancers per 1000 women by use of an endometrial cytologic technique. This report is derived from a multicenter clinical trial of estrogen and progestin replacement therapy in perimenopausal and postmenopausal women. We have evaluated the endometrial biopsy results from 801 asymptomatic perimenopausal or postmenopausal women to determine prospectively the incidence and/or prevalence of abnormal endometrial histologic characteristics.

Material and methods

Volunteers. The women recruited for this study were all volunteers who were interested in hormonal replacement for relief of menopausal symptoms. The protocol and informed consent had been approved by the local institutional review boards at each clinical site. Each volunteer was apprised of the reason for the study and had signed an informed consent form before any investigative procedure. Because of the nature of the study, all volunteers were required to have an intact uterus and no evidence of estrogen-dependent neoplasia of either the breast or the reproductive tract. Volunteers also were excluded if there was evidence of drug or alcohol abuse, thrombophlebitis, or thromboembolic disorders. Serum follicle-stimulating hormone levels were obtained on all women who were <45 years old or who had equivocal clinical symptoms. A serum follicle-stimulating hormone level >100 mIU/ml and a luteinizing hormone level >75 mIU/ml were indicative of the postmenopausal state. All volunteers who had received exogenous sex hormones were required to have discontinued all hormonal medication for at least 60 days before enrollment (biopsy) in the study.

Endometrial biopsy. Each volunteer underwent a screening endometrial biopsy with the sample being obtained from the anterior and posterior uterine walls. The endometrial samples were immediately placed in 4% paraformaldehyde in phosphate buffer containing 0.25% cetylpyridinium chloride. All samples were then shipped to the Electron Microscopy Center of the Department of Anatomy at the College of Medicine, University of South Alabama, for histologic evaluation by two of the authors (W.W.W. and E.A.D.) using light microscopy. Each biopsy specimen was then categorized by the same two authors using the following criteria:

1. Atrophy. This broad category includes the patterns of marked atrophy, inactive endometrium of perimenopausal type, posthyperplastic atrophy, and progestin-suppressed endometrium. Some epithelial snouts and vacuoles may be present.
2. Proliferative endometrium. The endometrium shows discernible evidence of stimulation—small,

tubular glands, fine cytoplasmic vacuoles, and columnar surface epithelium, with ciliation and mitoses in epithelial cells and/or stroma.

3. Secretory endometrium. Glands may show subnuclear glycogen vacuoles with more advanced secretory change; glands are tortuous with luminal secretion. Stroma is hypertrophic with periarteriolar cuffing. Mitoses are rare or absent.
4. Hyperplastic endometrium. A spectrum of glandular patterns (cystic, outpouching, or complex), crowding (glands at the expense of stroma), and cytologic abnormality characterizes the diagnostic subcategories of cystic, adenomatous, and atypical hyperplasia.
5. Insufficient for definitive diagnosis.

Results

For the purposes of this analysis we used the pathologic diagnosis of each sample by one author (E.A.D.). Screening endometrial biopsies were performed in 801 women. Seven hundred thirty-two were enrolled in the hormone replacement study as of the cutoff date, Dec. 31, 1988. The enrolled women were 52.1 ± 5.7 years old (mean ± SD, range 37 to 75) and weighed 148.4 ± 29.1 pounds (mean ± SD, range 87 to 274) (Table I). Postmenopausal status, defined as at least 12 months since a menstrual period, was assigned to 49.2%; perimenopausal status (<12 months since a menstrual period), to 48.9%, and unrecorded menopausal status, to 1.9%. The group comprised 90.4% white women, 4.4% black women, 4.5% Hispanic women, and 0.7% women of other ethnic groups.

Results of the 801 screening biopsies are shown in Table II and in Fig. 1. Histologic evidence of atrophy was noted in 373 (46.9%). Proliferative endometrium was found in 133 (16.7%), while secretory changes were shown in 54 (6.8%). It was not possible to relate biopsy results to previous use of sex hormones.

Hyperplastic endometrium was found in 41 (5.2%), and an additional 5 women had atypical cellular changes necessitating a repeat biopsy. The two hyperplasia groups were further classified into simple (31, 3.9%), cystic (9, 1.13%), and adenomatous (1, 0.13%). The five atypical cellular findings that necessitated further evaluation resulted in diagnosis of one case of well-differentiated adenoacanthoma.

Screening biopsy specimens contained insufficient tissue for a diagnosis in 195 (24.50%). These specimens contained endocervical tissue or mucus only, or fragments of endometrium too minute to evaluate, or no discernible tissue at all.

Polyps were found in 9 samples (1.13%). All these specimens except one also contained sufficient additional endometrium for another diagnosis, including atrophy, proliferative endometrium and cystic hyperplasia. These biopsy specimens have been classified according to these diagnoses. Other miscellaneous findings included acute endometritis (2 patients).

Table I. Demographic characteristics of women enrolled in this study

Total	732
Age (yr)	
Mean ± SD	52.1 ± 5.7
Range	37-75
Weight (lb)	
Mean ± SD	148.4 ± 29.1
Range	87-274
Perimenopausal (%)	48.9
Postmenopausal (%)	49.2
Ethnic group (%)	
White	90.4
Black	4.4
Oriental	0.4
Hispanic	4.5
Other	0.3

Table II. Endometrial histology in 801 perimenopausal and postmenopausal women without symptoms

Histology	*No.*	*%*
Atrophic	373	46.9
Proliferative	133	16.7
Secretory	54	6.8
Hyperplasia		
All	41	5.2
Simple	31	3.9
Cystic	9	1.1
Adenomatous	1	0.1
Cellular atypia	5	0.6
Insufficient tissue	195	24.5

Comment

It is axiomatic in gynecology that appropriate diagnostic studies should be performed in women with abnormal uterine bleeding. This is particularly relevant in postmenopausal women; any bleeding should arouse suspicion of neoplasia.

This study addresses the issue of endometrial biopsy in asymptomatic perimenopausal and postmenopausal women before initiation of hormone replacement therapy. No large-scale histologic study addressing this issue has been published.

Endometrial carcinoma was found in 1 of 801 asymptomatic women for an incidence of 1.3 per 1000. The expected incidence of endometrial carcinoma is approximately 1 to 2 per 1000 woman years.[9]

Our yield is lower than that of Koss et al.,[9] who discovered 16 cases of endometrial carcinoma in 2586 women on first screening, for a rate of 6.2 per 1000. However, 21.8% of this group were estrogen users.

Furthermore, this group was solicited for the purpose of cancer screening; thus self-selection bias may have increased the risk of this population. In fact, Koss et al. believed that some patients concealed symptoms of abnormal bleeding. In contrast, our study group was solicited for entry into a hormone replacement study, and the women were required not to have any abnormal bleeding. Thus our population may not be comparable with that in Koss's study.

Approximately three fourths of the women in our study had either atrophic endometrium or insufficient tissue on biopsy. Most of these insufficient specimens were thought to result from endometrium so atrophic that an adequate sample could not be obtained. It is therefore unlikely that significant endometrial pathologic abnormality was missed.

Proliferative endometrium was found in 16.7% of our population, and secretory changes were shown in 6.8%. We were not able to determine whether these results reflect endogenous or exogenous hormone action. However, all volunteers were free of hormonal medication for a minimum of 60 days before biopsy. Because 49% of the women were perimenopausal, it would not be unexpected to find evidence of endogenous stimulation of the endometrium.

We conclude from this study that the incidence of endometrial carcinoma in perimenopausal and postmenopausal women without symptoms is low; therefore, routine biopsy in such a group is a low-yield procedure. Some would conclude that biopsy before hormone therapy is indicated in spite of the low rate of positivity, a view espoused by two of the authors (W.W.W. and E.A.D.). Others, including two other authors (D.F.A. and K.M.S.), would conclude that biopsy in women without symptoms is unwarranted, in view of the low rate of abnormality in this group and the early onset of bleeding in women with endometrial neoplasia. This latter approach has also been recommended by the Canadian Task Force.[6] We all agree that women with symptoms require appropriate investigation. It should be reemphasized that conclusions that are based on our data apply only to women without symptoms. We do believe that all women receiving unopposed estrogens need yearly endometrial sampling. This should be a very small group of women, on the basis of current recommendations to use combined estrogen-progestin replacement.

The following physicians are the principal investigators who participated in this study: Eli Y. Adashi, Baltimore; David F. Archer, Pittsburgh; Bruce R. Carr, Dallas; Fred D. Fingerhut, Phoenix; Phillip C. Galle, Springfield, Ill.; David S. Horner, Charlotte, N.C.; David A. Iddenden, Philadelphia; Edward L. Klaiber, Worcester, Mass.; Clayton H. McCracken, Jr., Billings, Mont.; Daniel R. Mishell, Jr., Los Angeles; Lila E. Nachtigall, New York; M. E. Quigley, San Diego; Salvatore Rivi, Myrtle Beach, S. C.; Robert S. Schenken, San Antonio; Lawrence S. Seedman, Elkins Park, Pa.; Emil Steinberger, Houston; Eric M. Wall, Portland, Ore.; Lawrence E. Word, Lubbock, Tex.

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Discussion

DR. CECIL F. LANGFORD, Spartanburg, South Carolina (Official Guest). Dr. Archer and his colleagues are to be commended in accepting the challenge of a formidable and time-consuming study. The meticulous design of their research endeavor, the clarity of criteria for patient selection, the precise definitions of the histopathologic interpretations, and the analysis and collation of the findings provide us with valid criteria for understanding and clinical application of the results.

Certain observations seem worthy of comment. The fact that 46.8% of the biopsies produced atrophic endometrium and 24.5% of them produced insufficient tissue for diagnosis meant that 71.3% of the specimens could not be interpreted because of total absence or inadequacy of tissue obtained at biopsy. This will initially institute some concern over the accuracy of the study. Whereas the article states that both the anterior and posterior walls of the endometrium were sampled, it is not clear what type of instrumentation was used in obtaining the tissue. This is of some importance because most gynecologists and indeed pathology laboratories have their own preference as to the instruments found to be more efficacious in their hands for adequate sampling (i.e., Vabra aspiration, Gravely jet wash, Novak aspiration curette, Pipelle aspiration cannula). The authors did not indicate which technique was used in this study.

The observation that adenocarcinoma of the corpus uteri may in some instances be focal in its origin and its distribution might again raise the question of adequate sampling. In spite of this, current opinion suggests that endometrial sampling by most modern techniques, in contrast to results of older studies, results in a higher degree of accuracy and adequate assessment of the endometrial surface epithelium.

Without question, the most reassuring result of the study in 796 women is that only one case of overt adenocarcinoma was found. Four other biopsy specimens demonstrating cellular atypia required further evaluation. We believe that hyperplasia without atypia is not a premalignant lesion; therefore these four cases of atypia must be viewed with concern because 20% of cases with atypical adenomatous hyperplasia are associated with an occult or unrecognized well-differentiated adenocarcinoma of the endometrium.

Endometrial cancer manifests early with vaginal bleeding and has a 5-year survival rate of 75%; one wonders if the results would be improved by routine screening. Williamson (personal communication, 1990) found only three cases of atypical epithelia in 100 high-risk patients (overweight diabetic patients considered to be at high risk).

Continuous unopposed estrogen therapy is believed by many to be associated with if not the causative factor in certain cases of adenocarcinoma of the uterine corpus. Current trends that include the use of a combination preparation including estrogens and a progestogen not only are more logical from a physiologic standpoint but also will reduce the population considered at risk because they do not periodically receive a progestational agent to induce periodic shedding of the endometrium. This is alluded to by Dr. Archer in that in the future there will be a decreasing patient population requiring periodic endometrial sampling.

Numerous publications in the lay and medical press provide convincing evidence of the value of postmenopausal hormonal therapy. These include its impact on the reproductive tract, skin and its appendages, and urinary tract epithelium, the maintenance of urinary continence, and, paramount to all of the above, prevention or amelioration of the crippling effects of osteoporosis. In the same publication the patient and the gynecologists are warned that such therapy may be carcinogenic. Studies such as this one should minimize or at least put into perspective many of these concerns. Added to the acceptance by the patient will be the absence of vaginal bleeding; we find such bleeding to be a major negative factor in patients' attitudes toward postmenopausal therapy.

I agree that the yield of neoplasia in this study is so low that screening endometrial biopsy is not justified in perimenopausal and postmenopausal women without symptoms.

In conclusion, the authors have comprehensively assessed the endometrium in a large group of perimenopausal and postmenopausal women. I would pose only one question: Do you have a follow-up plan designed to assess the subsequent evaluation of endometrial changes, particularly preneoplastic or neoplastic lesions in the patient population?

DR. WALLACE C. NUNLEY, JR., Charlotte, North Carolina. Dr. Archer, five patients had endometrial histologic abnormalities, and presumably these patients might respond to a progestin challenge. Would you consider, instead of a routine endometrial biopsy, a progestin challenge? If that were positive, it would select a group of patients in whom biopsy might be indicated, in contrast to the 75% of patients with inadequate tissue or atrophic endometrium, in whom you probably would not get a positive progestin response.

DR. JAROSLAV F. HULKA, Chapel Hill, North Carolina. Vaginal ultrasonography has recently been documented to be a fairly good screen for endometrial changes. Would you care to comment or speculate on the future role of the combination of biopsy and vaginal ultrasonography?

DR. R. DON GAMBRELL, JR., Augusta, Georgia. I would like to point out a study of a much smaller number of patients reported in the AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY in 1984 by Hanna et al., in which they gave a challenge of 100 mg progesterone in only 30 patients. Seventeen percent had withdrawal bleeding. They performed biopsies in all these women. The ones who did not have withdrawal bleeding had atrophic, inactive endometrium, and in 60% of the ones who had withdrawal bleeding there was some degree of endometrial hyperplasia.

DR. ARCHER (Closing). Dr. Langford pointed out the issues confronting us in terms of sensitivity and the concerns we had about sampling techniques. We have follow-up plans. As I have mentioned, 796 women were screened and 732 were enrolled in this study. Dr. McIntyre-Seltman and I are currently reevaluating these data because they were put into six different treatment arms with estrogen-progestin replacement therapy. Endometrial biopsies were carried out after 6 and 12 months of treatment at the appropriate times in the cycles. We are evaluating those data at the present time.

One of our concerns is whether we should be doing progesterone challenge tests. At the present time we are uncertain as to its efficacy. I feel that if I am going to place a woman on a hormone replacement therapy regimen, I will go ahead and do so, arguing that I am going to use both an estrogen and a progestin and thereby circumvent significant endometrial changes. I have used the progesterone challenge test in the past. Dr. Gambrell has published on this very issue in terms of the use of progestins in early perimenopausal and postmenopausal women.[1] I admit that it will sort out those women who have atrophic endometrium from those who have some degree of stimulation. I don't have any data to support the fact that a significant group of women with bleeding may be more at risk for endometrial adenocarcinoma or hyperplasia.

We have been doing vaginal ultrasonographic examinations on patients who are on hormone replacement therapy regimens as a part of clinical studies under various protocols. I am well aware of the data from Europe, in postmenopausal women who have experienced vaginal bleeding, that an endometrial thickness >5 mm by ultrasonography will identify a group of women likely to have some abnormality of the endometrium.[2] On the other hand, when the endometrial thickness is <5 mm, one would expect to find an atrophic endometrium. We have not done enough studies in patients to find a correlation. We are doing ultrasonographic examination before endometrial biopsy in several clinical protocols in women who are receiving estrogen and progestins.

How good is ultrasonography in assessing the entire endometrial cavity? I am a neophyte in this area, and I feel very inadequate, thinking that I might miss something, if I look at a vaginal sonogram that I have obtained in terms of endometrial thickness. This goes back to the same point that adenocarcinoma can be focal, and we may be missing something with these biopsies. The use of vaginal ultrasonography is interesting in the evaluation of the endometrium, but I am not sure of its clinical validity.

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Assessment of Kegel pelvic muscle exercise performance after brief verbal instruction

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Forty-seven women had urethral pressure profile determinations performed at rest and during a Kegel pelvic muscle contraction, after brief standardized verbal instruction. Twenty-three (49%) had an ideal Kegel effort—a significant increase in the force of urethral closure without an appreciable Valsalva effort. Twelve subjects (25%) displayed a Kegel technique that could potentially promote incontinence. Age, parity, weight, estrogen deprivation, prior continence surgery or hysterectomy, and passive urethral function did not predict a successful effort. We concluded that simple verbal or written instruction does not represent adequate preparation for a patient who is about to start a Kegel exercise program. (Am J Obstet Gynecol 1991;165:322-9.)

Key words: Kegel exercises, pelvic muscle exercises, urinary incontinence

In the late 1940s, the gynecologist Arnold Kegel[1] proposed the use of pelvic muscle exercises to improve the function and tone of the pelvic floor after childbirth, to correct mild anatomic defects such as early cystocele and rectocele, and to treat stress urinary incontinence. Kegel exercises represent the voluntary contraction and relaxation of the levator ani muscle (principally the pubococcygeus and puborectalis portions), which supports the vagina, bladder, and urethra and which contributes to the skeletal muscle component of the urethral sphincteric mechanism. The goal of Kegel exercises in the treatment of urinary incontinence is to increase the strength and endurance of these muscles, thereby enhancing the force of urethral closure under certain conditions, such as with a sudden increase in abdominal pressure. Implicit to our understanding of the physiologic basis of Kegel exercises is that a properly performed contraction should increase the force of urethral closure.

Some women seem unable to contract the proper muscles when given verbal instruction about Kegel exercises; they often perform a Valsalva maneuver or contract the gluteal and thigh muscles exclusively or in combination with contraction of the levators. Kegel noted that many women are unaware of their pelvic

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muscle function and require some method of performance feedback to successfully contract the proper muscles. To assist patients by providing such biofeedback, he developed the perineometer, a pneumatic device consisting of a vaginal probe connected to a manometer. While vaginal pressure measurements, electromyographic activity, and digital palpation of pubococcygeus muscle tone have all been advocated as biofeedback techniques to facilitate the initiation of a Kegel exercise program, most patients start (and finish) such a program with only brief written or oral instructions. The American College of Obstetricians and Gynecologists' patient education pamphlet on urinary incontinence instructs patients to "stop the flow of urine in midstream without tightening or tensing your leg or stomach muscles." The aim of this study was to assess the effect of such a simple brief verbal instruction on urethral sphincteric function during a Kegel contraction. A secondary aim was to attempt to identify clinical or urodynamic characteristics that might predict an effective Kegel contraction effort.

Subjects and methods

The study population included 47 consecutive subjects who presented to the Gynecologic Urodynamic Laboratory at the Medical College of Virginia Hospitals for evaluation. Their mean age was 53.6 years (range 23 to 83 years) and their mean parity was 2.5 (range 0 to 8). Twenty-eight subjects (60%) had urinary incontinence as the major complaint. Of those who were incontinent, 14 had pure genuine stress incontinence, four had pure detrusor instability, six had mixed incontinence (genuine stress incontinence plus detrusor instability), and four had pure urethral instability. Nineteen subjects (40%) did not complain of incontinence. Five of these had vaginal vault prolapse, four had emptying phase defects, five had sensory urgency without incontinence or motor instability, and five were being evaluated 6 months after successful continence surgery. Eighteen subjects (38%) had prior continence surgery, 27 (60%) had a prior hysterectomy, 7 (15%) had severe pelvic organ prolapse (2 of whom also had detrusor instability), and 7 (15%) were hypoestrogenic.

All subjects underwent a standardized urodynamic evaluation that included uroflowmetry with postvoid residual urine volume measurement, retrograde provocative multichannel water urethrocystometry with the use of microtip transducer catheters, passive and dynamic urethral pressure profiles, resting and stressed urethral axis determinations, and direct visualization testing for fluid loss. Specific details of the urodynamic methods have been described in detail previously.[2-4] All subjects had a negative bacterial urine culture (<100 CFU/ml) before being scheduled for urodynamic testing and none had pyuria at the time of the testing.

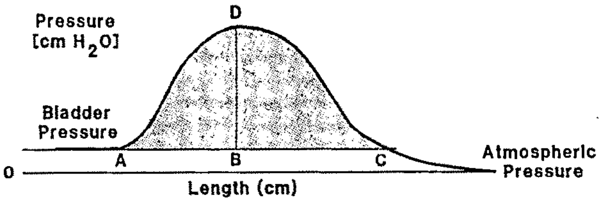

Fig. 1. Measurements derived from the urethral pressure profile. *Line A to C* is total functional urethral length; *line B to D* is maximum urethral closure pressure. *Shaded area* represents total urethral pressure profile area. Mean urethral closure pressure is calculated by dividing profile area by functional urethral length.

After each subject had completed the standard passive and dynamic urethral pressure profile (at a bladder volume of 300 ml and a transducer withdrawal speed of 0.5 mm/sec), a passive urethral pressure profile was repeated at a catheter speed of 5.0 mm/sec. Each subject was then asked to "contract the muscles you would use if you were trying to keep from losing your urine or if you were trying to stop your stream after you had started to urinate." Once the subject had acknowledged that she understood the instructions and had practiced a contraction, she was allowed to relax for a short time. It was then explained that she would be asked to contract the same muscles on command and to hold the contraction until she was told she could relax. Another urethral pressure profile was then performed, with the patient having been asked to start a contraction just before catheter withdrawal was started and being allowed to relax as soon as the urethral closure pressure became negative. This profile was designated the Kegel urethral pressure profile. The catheter withdrawal speed of 5.0 mm/sec allowed all Kegel profiles to be completed in <8 seconds, a period of time for which most patients could sustain a single contraction effort.

Pressures recorded with a physiologic recorder during the profiles included the vesical, urethral, abdominal (vaginal), detrusor, and urethral closure pressures. Parameters measured and analyzed from the urethral pressure profiles included the functional urethral length, maximum urethral closure pressure, total profile area (determined with a polar compensation planimeter), and mean urethral closure pressure (profile area/functional urethral length). These measurements are illustrated in Fig. 1. Urodynamic techniques and measurements, terms, and diagnostic criteria conform to the recommendations of the International Continence Society.[5]

A subject was considered to have had an effective Kegel effort if the Kegel urethral pressure profile area was ≥120% of the passive urethral pressure profile area or if both the Kegel maximum urethral closure pressure and the mean urethral closure pressure were

Table I. Comparison of changes from passive to Kegel urethral pressure profile according to Kegel effort group assignment

Change	*Effective effort (n = 28)*	*Ineffective effort (n = 19)*	*p Value*
Functional urethral length			0.04*
Absolute (cm)			
Mean ± SE	0 ± 0.9	−2.0 ± 0.7	
Range	−11-14	−8-6	
Percentage			
Mean	+1	−9	
Range	−41-58	−28-18	
Maximum urethral closure pressure			0.0000003*
Absolute (cm H_2O)			
Mean ± SE	16.5 ± 2.4	−1.5 ± 1.4	
Range	−4-56	−16-6	
Percentage			
Mean	+70	−6	
Range	−6-450	−53-33	
Area			0.00000009*
Absolute (mm × cm)			
Mean ± SE	223.3 ± 35.3	−53.3 ± 19.8	
Range	−38-888	−222-102	
Percentage			
Mean	+82	−17	
Range	−14-779	−70-18	
Mean urethral closure pressure			0.00000005*
Absolute (cm H_2O)			
Mean ± SE	9.4 ± 1.3	−1.0 ± 0.7	
Range	1-36.1	−7.4-6.1	
Percentage			
Mean	+80	−10	
Range	8-633	−60-28	

*Kruskal-Wallis one-way analysis by ranks.

≥120% of the passive maximum and mean urethral closure pressure. Subjects who did not fulfill either of these criteria were considered to have had an ineffective Kegel effort. The degree of Valsalva effort was assessed by measuring the changes in vesical and vaginal pressures. The Valsalva effort was considered excessive if the rise in vesical pressure exceeded 15 cm H_2O during the Kegel urethral pressure profile.

Categoric data were analyzed for significant differences with the χ^2 test with Yates' correction. Quantitative data were analyzed with analysis of variance and the Kruskal-Wallis one-way analysis by ranks for between-group comparisons, the paired *t* test for passive and Kegel urethral pressure profile comparisons, and simple linear regression analysis for vesical and vaginal pressure correlations.

Results

Twenty-eight subjects (60%) had an effective Kegel effort and 19 (40%) had an ineffective effort. With a Kegel contraction the former group increased the maximum urethral closure pressure (mean ± SE) from 35.0 ± 3.2 to 51.5 ± 3.7 cm H_2O ($p = 0.000001$), the total profile area from 449 ± 54 to 671 ± 68 mm × cm ($p = 0.0000008$), and the mean urethral closure pressure from 18.7 ± 2.0 to 28.1 ± 2.4 cm H_2O ($p = 0.0000004$). In contrast, the group with an ineffective effort had no significant increases in any of these parameters with maximum urethral closure pressure values of 32.2 ± 3.5 and 30.7 ± 3.6, total profile areas of 440 ± 53 and 386 ± 58, and mean urethral closure pressure values of 17.4 ± 1.7 and 16.4 ± 2.0 for passive and Kegel profiles, respectively. There were no significant changes in functional urethral length in either the effective or ineffective group. Table I compares the changes between the passive and Kegel profiles for the two groups. As expected, there were highly significant differences between the two groups for the changes in all profile measurements.

As demonstrated in Table II, there were no significant differences between the effective and ineffective Kegel effort groups with respect to age, parity, weight, or any of the passive profile measurements. Likewise, there were no significant differences between the groups with respect to the prevalence of prior continence surgery, prior hysterectomy, severe pelvic organ prolapse, hypoestrogenism, or genuine stress incontinence. When the ability to perform an effective Kegel contraction was compared to urodynamic diagnosis, no clear pattern was seen; 57% of patients with pure genuine stress incontinence (8/14), 67% with mixed incontinence (4/6), 75% with pure unstable urethra (3/4), 33% with pure detrusor instability (1/3), 60% with vaginal vault prolapse (3/5), 100% with emptying phase dysfunction (4/4), and 50% with no abnormal diagnosis (5/10) were able to generate an effective Kegel effort.

Twelve subjects (26%) had increases in vesical pressure >15 cm H_2O during the Kegel contraction, 5 in the effective effort group and 7 in the ineffective effort group (difference not statistically significant). The change in vesical pressure (mean ± SE) for those with excessive Valsalva effort was 39.8 ± 12.5 cm H_2O in the effective Kegel group and 21.6 ± 4.6 cm H_2O in the ineffective Kegel group. The respective changes for those without excessive Valsalva effort were 5.9 ± 2.1 and 6.5 ± 1.2 cm H_2O. Four of the five subjects with improvement in urethral measurements after Valsalva effort had either prior successful continence surgery or severe pelvic organ prolapse, conditions in which Valsalva stress is known to enhance urethral closure because of physical obstruction.[6, 7] As anticipated, there was a highly significant correlation between the changes in vesical and vaginal pressures during the Kegel effort ($r = 0.8306, p < 0.00001$).

Comment

This study has demonstrated that 60% of the 47 subjects were able to generate an effective increase in the force of urethral closure after brief standardized verbal instruction. However, 5 of 28 subjects with an effective Kegel effort achieved this with an appreciable increase in abdominal and vaginal pressure. Thus only 23 of 47 subjects (49%) had an ideal Kegel effort, that is, a significant increase in urethral pressure without a concurrent increase in vesical and abdominal pressure. Moreover, we were unable to identify any historic or urodynamic parameter that reliably identified subjects who were likely to have either an effective or an ineffective Kegel effort. These included age, parity, weight, prior continence surgery, prior hysterectomy, severe pelvic organ prolapse, any parameter on a passive urethral pressure profile, and any urodynamic diagnostic category.

It is recognized that our criteria for an effective Kegel contraction were arbitrary, but this was necessary because no criteria have been previously established. In defense of our criteria, it should be emphasized that very few subjects in either group came close to our cutoff values. For example, only one subject in the ineffective group increased the total profile area by >8% and 13 of 19 subjects in this group actually decreased the profile area with the Kegel effort. In contrast, only 4 subjects in the effective group had <20% increase in total profile area; they had increases in maximum and mean urethral closure pressures that averaged 31% and 46%, respectively. The lack of change in profile area in these subjects was due to a decrease in functional urethral length that averaged 28%. We thought this resulted from urethrovesical junction movement (elevation) away from the urethral transducer during the Kegel profile, the net effect of which would be an increase in the speed of withdrawal of the urethral transducer relative to the paper speed of the recorder. We attempted to have the Kegel contraction established before catheter withdrawal to obviate this technical problem; however, this goal obviously was not realized in these four subjects.

Table II. Clinical and passive urethral pressure profile comparisons between effective and ineffective Kegel effort groups

	Effective effort (n = 28)	*Ineffective effort (n = 19)*
Age (yr)		
Mean ± SE	52.3 ± 2.8	55.7 ± 3.5
Range	23-74	27-83
Parity		
Mean ± SE	2.4 ± 0.3	2.7 ± 0.4
Range	0-6	0-8
Weight (kg)		
Mean ± SE	80.4 ± 6.2	66.3 ± 3.1
Range	55-200	46-104
Functional urethral length (mm)		
Mean ± SE	23.5 ± 0.8	23.5 ± 1.5
Range	16-32	7-35
Maximum urethral closure pressure (cm H_2O)		
Mean ± SE	35 ± 3.2	32 ± 3.5
Range	11-68	4-68
Area (mm × cm)		
Mean ± SE	449 ± 55	440 ± 53
Range	114-1220	14-1014
Mean urethral closure pressure (cm H_2O)		
Mean	18.7	17.4
Range	5.3-45.0	2.0 to 32.3
Continence surgery (No.)	10 (37%)	8 (42%)
Hysterectomy (No.)	15 (56%)	12 (63%)
Severe prolapse (No.)	3 (11%)	4 (21%)
Hypoestrogenic (No.)	3 (11%)	4 (21%)
Genuine stress incontinence (No.)	12 (43%)	8 (42%)

We also recognize that the small number of subjects in some of our groups limits the power of any conclusion regarding the lack of effect of certain factors on the ability to perform an effective Kegel contraction. However, it is clear that none of these factors guarantees either success or failure. Fig. 2 contains tracings from three subjects that illustrate these points vividly. Part *2A* is a passive and Kegel urethral pressure profile from a 62-year-old, para 5 woman with recurrent mixed incontinence, a prior hysterectomy, and a Marshall-Marchetti-Krantz procedure. She was post-

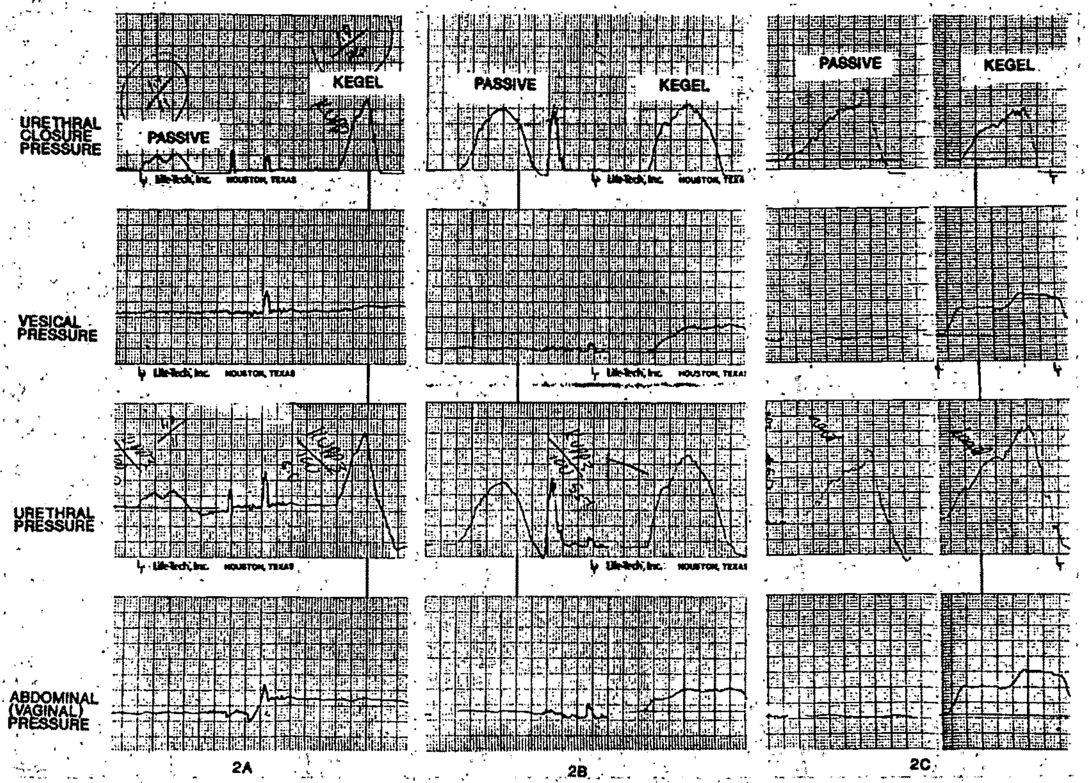

Fig. 2. Tracings of passive and Kegel urethral pressure profile of three subjects. See Comment section for details. **2A,** 62-year-old, multiparous subject with effective Kegel effort; **2B,** 27-year-old nulliparous subject with ineffective Kegel effort and significant Valsalva effort; **2C,** 52-year-old multiparous subject with genuine stress incontinence whose Valsalva effort with Kegel attempt resulted in decrease in force of urethral closure.

menopausal for 14 years but had never received estrogen replacement therapy. The passive profile demonstrated a markedly hypotonic urethra with a functional urethral length of 17 mm and a maximum urethral closure pressure of 11 cm H_2O. In spite of a variety of factors that led us to intuitively believe that she would be unable to display an effective Kegel contraction, she was able to increase the total profile area, maximum urethral closure pressure, and mean urethral closure pressure by 186%, 318%, and 170%, respectively. In contrast, part *2B* is a tracing from a 27-year-old nulliparous woman with sensory urgency, normal ovarian function, and no prior urogynecologic survey. In spite of characteristics that we believed would predict an effective Kegel effort, the only effect of her Kegel effort was a 15 cm H_2O increase in vaginal and vesical pressure with no appreciable change in urethral closure pressure.

Part *2C* illustrates a potential danger of incorrectly performed Kegel exercises. This tracing is from a 52-year-old, gravida 2 woman with primary pure genuine stress incontinence. Her Kegel effort was accompanied by a significant Valsalva effort with an increase in vaginal and vesical pressure of nearly 30 cm H_2O. This increase in abdominal pressure was transmitted only partially to the urethra with the net effect being a decrease in total profile area, maximum urethral closure pressure, and mean urethral closure pressure of 32%, 28%, and 11%, respectively. Thus, instead of improving the force of urethral closure, the Kegel technique actually had the opposite effect. Further, a structured program of regular repetition of this technique might actually lead to progression of the anatomic and functional defect that is the cause of incontinence in this woman.

This study offers no insight into the predictive value of the Kegel urethral pressure profile. We do not know if an effective Kegel effort by a woman predicts successful control of incontinence if she pursues a structured Kegel exercise program, nor do we know if ineffective Kegel efforts can be made effective through reeducation and the use of biofeedback. Finally, we do not know if an improvement in the force of urethral closure with a Kegel contraction will correlate positively with an improvement in urinary control. These are all topics of ongoing inquiry in our unit.

What does seem obvious from this study is that simple verbal or written instruction does not represent adequate preparation for a patient who is about to pursue a Kegel exercise program. While approximately half of women are able to perform an effective contraction, half are not and as many as one fourth may adopt a technique that could potentially promote the defect responsible for their incontinence. Some form of initial assessment, instruction, and feedback such as pubococcygeal and abdominal muscle palpation with verbal feedback, the use of differential (upper vagina reflecting abdominal pressure and lower vagina reflecting levator tone) vaginal pressure biofeedback, or the use of electromyographic biofeedback seems desirable. Saving such techniques only for Kegel failures needlessly postpones potentially effective therapy for a significant number of women who suffer from incontinence. In addition, many women who feel that they have failed their initial trial with a Kegel exercise program will refuse to accept any additional physiotherapy.[8]

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Discussion

DR. A. CULLEN RICHARDSON, Atlanta, Georgia. I congratulate the authors on a very carefully done study with carefully recorded exact data. It is encouraging to see attention returned to the striated muscle in the pelvis. We need to understand more about its role in pelvic support in general, as well as in urinary continence.

My principal criticism of this article is that at this point I consider it to be an incomplete study. This is an excellent first step in a much-needed investigation of Kegel exercises, but the data contained in this study, although excellent, are not sufficient to permit significant conclusions.

The study lacks a control group of perfectly normal nulliparous and parous patients. Patients presenting to a urodynamics clinic are hardly a representative patient population. These results are not applicable to the general population.

The authors concluded that simple verbal instruction does not represent adequate preparation for a patient who is about to start a Kegel exercise program. However, they did not report how many of those who had no change in urethral closure pressure were eventually taught to sustain good levator contractions.

There were variables for which there were no corrections.

At the beginning the authors made an assumption: "Implicit to our understanding of the physiologic basis of Kegel exercises is that a properly performed contraction should increase the force of urethral closure." This would be true only in patients with perfectly normal anatomy, which few of their patients had.

Potentially, there are two anatomic variables that must be controlled for that were not addressed in this article. As this is essentially a test of isolated striated muscles, normal function would require, first, structural integrity of the muscle itself and its attachment and, second, intact neurologic innervation of the particular muscle being studied.

The effect on urethral pressure with voluntary contraction of the pelvic floor muscle is exerted through three groups of striated muscle fibers. First and most prominent is the pubovaginal portion of the pubococcygeus muscle (fibers of Luschka). Second, there are the very thin, roughly circular striated periurethral fibers that terminate in the musculus transversus perinei profundus of the urogenital diaphragm. Third are the fibers in the musculus transversus perinei profundus that go over the top of the urethra just as it passes under the symphysis (so-called compressor urethra muscle). In the case of the pubovaginal fibers of Luschka, the force is not on the urethra itself but is transmitted by way of the pubocervical fascia within which the urethra is embedded.

If these striated muscle fibers are detached from the vagina or the paraurethral fascia is broken, then even with good pubococcygeus contraction there would be no effect on urethral closure pressure. As the authors continue their study, there should be a control for pubococcygeus contractions, with fine-wire electromyography, to identify those patients who in spite of pubococcygeal contraction show no change in urethral closure pressure. I am sure these patients exist.

Even in patients whose muscles are intact, there can be a problem with neurologic innervation. This of course can extend from the cerebral cortex down through the cord and then through the peripheral nerves to the myoneural junction. The assumption in the study seemed to be that all of the problems were at the level of the cerebral cortex—failure to understand the instructions.

There is increasing evidence that many patients with genital support problems with or without bladder dysfunction manifest weakness in the pelvic floor striated muscle as a result of deficient or damaged neurologic innervation—a problem not particularly affected by exercise.

Gilpin et al.[1] and Smith et al.[2,3] in Manchester, England, Snooks et al.[4] in London, and Constantinou and Govan[5] in this country have studied and are studying this in depth. They have found some women whose problem is in the peripheral nerves at the spine as a result of bony deformities such as are seen with spondylitis, many in the pudendal nerves as a result of stretch injury during parturition, and some with stretch injury within the muscle itself.

This becomes even more complicated when one considers that the pelvic floor striated muscle contains both slow- and fast-twitch fibers. The slow-twitch fibers, which are quite prominent in the pelvic floor muscles, maintain some constant involuntary tone, much as is the case with the external anal sphincter. Undoubtedly the resting tone of these slow-twitch fibers in the levator group is the important factor in keeping the levator hiatus essentially closed in the patient with normal support.

Normal pelvic support is always a delicate interplay between the fibrous connective-type tissues and striated muscle tone—involuntary, reflex, and voluntary. Mechanically, the striated muscles not only serve as a backup mechanism to protect the overlying connective-type tissues (the fascias and ligaments) but are actually the mediators of certain functions, such as aiding in the closure of the bladder neck, as well as the anal sphincter. As yet we have no medical or good surgical treatments for inadequate striated muscle function.

We all tend to recommend Kegel exercises rather routinely. These would be ineffective, however, if the problem is one of neurologic change. Further, the authors have apparently identified a subset of patients in whom the exercises are not only ineffective but potentially deleterious.

Again I congratulate the authors on carefully recording these preliminary observations. My hope is that they will continue to study the pelvic floor striated muscle with normal controls, electromyographic monitoring of the pubococcygeus, and a careful record of the anatomic findings. Clearly their preliminary results confirm the need for much better understanding. Further, their study makes it clear that we need a better method of instructing patients in the "how to" of Kegel exercises.

I would ask them several questions at this time:

1. Do you have data that you did not include that would answer the questions posed?

2. What percent of patients were able to effect good levator contractions after more detailed instructions and/or some biofeedback?

3. At present, what have you found to be the best "how to" in instructing patients for Kegel exercises?

It is hoped that these authors, who possess the expertise to do such careful and exact studies, will continue their efforts and give us the much-needed additional data to clarify the preliminary findings reported here.

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Dr. Bump (Closing). With respect to the appropriateness of our study group, I would defend our population as exactly representative of the types of patients who are started on a Kegel exercise program. Therefore the information is directly applicable to the portion of the population that might start such a program. Kegel advocated his exercises for patients who needed to improve the function and tone of the pelvic floor, that is, after childbirth, with early cystocele and rectocele, and with incontinence. I should also mention that our incidence of ineffective Kegel effort corresponds with that of several other authors in the physiotherapy literature.

I don't doubt that some of our ineffective contractors were ineffective not because they did not understand which muscles to contract but because the muscles or the connective tissue attachments were rendered incapable of responding. However, our findings do not support this as the primary reason for failure, because subjects who were most likely to have such neuromuscular and anatomic compromise were able to perform just as well as subjects who had no evidence of such injury. Further, a significant proportion of subjects were not simply ineffective; nearly one quarter had efforts that were counterproductive.

With respect to Dr. Richardson's question of clandestine data that may address the questions as to the type and location of striated muscle involved in the continence mechanism, we are in the process of completing a study that confirms and expands on the findings in the studies of the groups he mentioned. One of our major research priorities is to develop techniques to study denervation injury and recovery. By way of drastic oversimplification I would simply state that there is no question in my mind that there are critical neuromuscular components to stress incontinence in women and that simple anatomic alterations do not explain the condition.

As to our eventual ability to teach ineffective contractors to become effective and the best way of providing instruction for Kegel exercises, I hope that our groups' efforts, under the primary direction of Dr. Jean Wyman, will allow us to answer that question, but the answer is 4 to 5 years away.

I think the worst way to instruct patients is to ask them to interrupt the urinary stream repeatedly during micturition. I have three primary aversions to this time-honored technique. First, many women, and most incontinent women, cannot interrupt the stream, and asking them to do what they are unable to do simply accentuates their feelings of helplessness and hopelessness. Second, I don't think that the way you improve the bladder's storage ability is by interfering with its emptying function. A contracting sphincter in the midst of normal micturition is a pathologic condition known as vesicosphincter dyssynergia, and I don't think we should encourage patients to practice this technique. Remember, we can entrain our bladders to adopt bad habits in addition to good ones. Finally, when the completely normal woman interrupts the urine stream, the urethral closure pressure becomes positive and flow stops several seconds before the detrusor muscle contraction is suppressed. This detrusor contraction against a voluntary outlet obstruction several times during every voiding episode may result in reflux and eventually upper tract injury.

Vulvar squamous cell carcinoma and papillomaviruses: Two separate entities?

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Vulvar squamous precancers (vulvar intraepithelial neoplasia) are associated with sexual factors, cigarette smoking, and human papillomaviruses. However, epidemiologic studies of invasive carcinoma of the vulva have produced conflicting evidence for these associations, in part because of a strong association with vulvar inflammatory disease (dystrophies) in older women. We analyzed a series of 42 vulvar invasive carcinomas for papillomavirus nucleic acids by deoxyribonucleic acid–deoxyribonucleic acid in situ hybridization and correlated their presence with age, smoking history, and morphologic type. The carcinomas were divided into well-differentiated, moderately and poorly differentiated, and intraepithelial-like growth patterns, the latter composed of nests of invasive neoplastic epithelium with preserved cell polarity, similar to intraepithelial disease. Of the lesions studied, 28% were human papillomavirus deoxyribonucleic acid–positive. Intraepithelial-like neoplasms segregated in women with a younger mean age (64 versus 73 years) than that of women with conventional squamous cell carcinoma and they more frequently had a history of cigarette smoking (88% versus 28%). Moreover, intraepithelial-like lesions contained human papillomavirus nucleic acids more frequently (67% versus 13%) when analyzed by in situ hybridization. These observations confirm the diverse nature of vulvar squamous cell carcinoma and may explain in part why conflicting results are obtained from studies investigating the role of sexual and viral factors in the genesis of vulvar cancer. They suggest that many invasive vulvar cancers may not be linked to papillomaviruses. (Am J Obstet Gynecol 1991;165:329-36.)

Key words: Vulvar carcinoma, human papillomavirus, vulvar intraepithelial neoplasia

Invasive carcinoma of the vulva is a rare disease with an incidence rate approximately one tenth that of its counterpart in the cervix.[1, 2] Traditionally the disease has predominated in women in their seventh and eighth decades and is rarely observed in women younger than age 30 years.[2] This pattern of disease has changed slightly in recent years, and investigators have focused on three components of this disease spectrum that may shed light on the increasingly younger age of women seen with vulvar neoplasia. The first is the oc-

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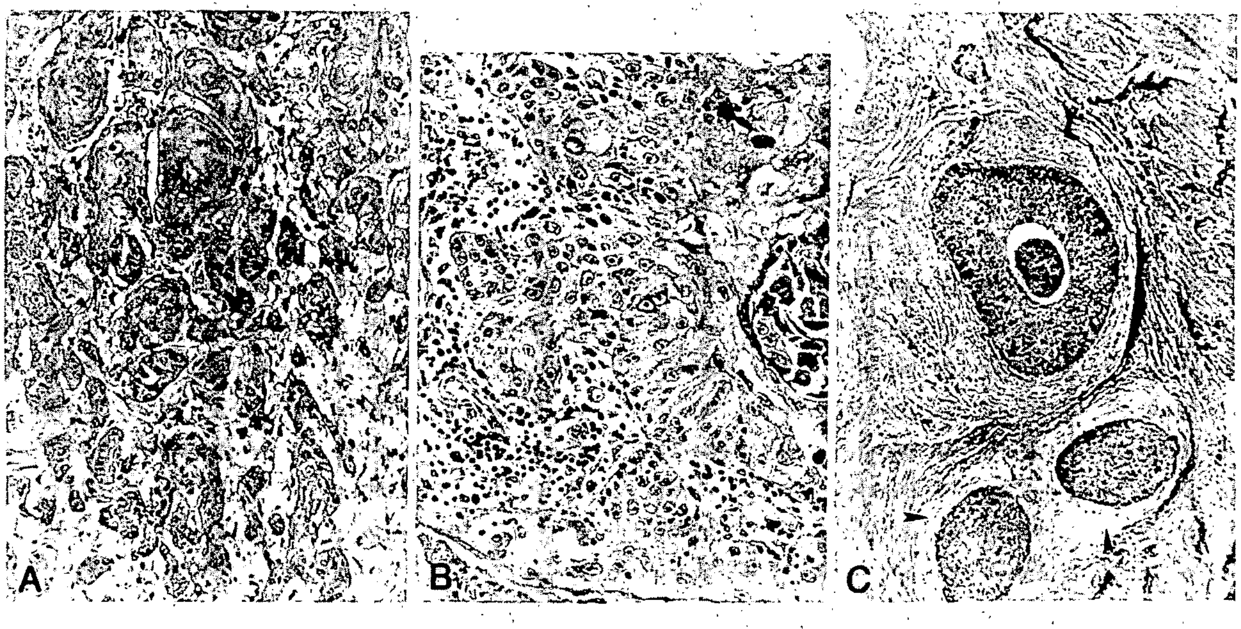

Fig. 1. Histologic subgroups studied. **A,** Well-differentiated keratinizing squamous cell carcinoma of the vulva; **B,** moderately to poorly differentiated squamous cell carcinoma of the vulva; **C,** intraepithelial-like squamous cell carcinoma of the vulva. Note the well-circumscribed nesting pattern of the tumor cells, similar to a precursor (vulvar intraepithelial neoplasia) lesion. The adjacent desmoplastic stromal changes *(arrows)* indicate that this is invasive cancer and not a tangentially sectioned vulvar intraepithelial neoplasia.

currence of vulvar precancers, or vulvar intraepithelial neoplasia, which are occurring more frequently in younger women and carry some risk of evolving into cancer as a function of increasing age.[3-6] The second is the human papillomavirus (HPV), which has been increasingly associated with cervical precancerous lesions and linked with vulvar intraepithelial neoplasms as well.[7] The third is the subset of squamous carcinomas, which are separated from their noninvasive counterparts by several decades, are infrequently associated with vulvar intraepithelial neoplasia, and appear to be more associated with vulvar inflammatory conditions than with sexually transmitted diseases.[8-10]

The potential discrepancy between sexual factors and vulvar cancer has been magnified by epidemiologic studies of vulvar carcinoma. Although vulvar neoplasia has been linked to sexual factors by some authors,[11-13] and a case-control study of vulvar carcinoma identified preexisting genital warts and smoking as singificant risk factors,[11] others have found little relationship between sexual factors and invasive vulvar carcinoma. Mabuchi et al.[9] failed to associate early age of coitus, first marriage and multiple miscarriages, or venereal factors with vulvar cancer.[9] They observed a relationship to prior history of vulvar inflammation and urogenital cancer, whereas the relationship to prior cervical cancer (six cases of 149) was of borderline significance.[9]

Those studies suggest that, from an epidemiologic perspective, vulvar cancer may encompass more than one distinct group. Additional evidence that vulvar carcinomas have a diverse pathogenesis comes from two morphologic studies that examine the epithelium adjacent to invasive vulvar cancers. Both Buscema et al.[8] and Zaino et al.[10] noted that in only approximately one fourth of invasive cancers was there evidence of a classic vulvar intraepithelial neoplasm (carcinoma in situ) in the adjacent epithelium. In contrast, more than one half of cases were associated with epithelial hyperplasia or atrophy and inflammation, with or without cytologic atypia (lichen sclerosis, squamous hyperplasia, atypia).[9, 10] These findings suggested that a significant proportion of vulvar carcinomas did not evolve from a long-standing precancerous lesion. It is important that cancer developed in only a fraction of women with chronic inflammatory diseases of the vulva.[14]

One recent study[15] corroborated the above observations, noting a variable detection rate of HPV nucleic acids in vulvar cancer. It is important that these authors[15] observed a strong relationship between smoking and women with HPV-related vulvar precancers versus squamous carcinomas, the majority of which had an associated vulvar intraepithelial neoplasia. Moreoever, another study has reported an absence of HPV nucleic acids in a small series of vulvar carcinomas associated with chronic inflammatory vulvar disease (lichen sclerosis) rather than with vulvar intraepithelial neoplasia.[16] From this it would appear that different subsets of cancers exist, and an association with vulvar

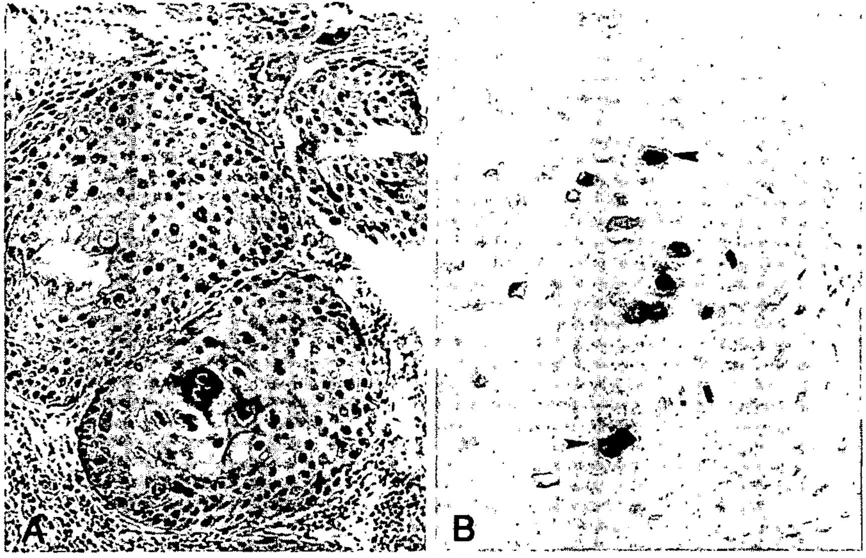

Fig. 2. Presence of HPV nucleic acids in intraepithelial-like invasive carcinoma of the vulva. **A,** Sharply demarcated nests of invasive carcinoma with preserved cellular polarity and basal palisaded nuclei. **B,** Serial section after hybridization with a biotinylated HPV type 16/18 mixed probe. Nuclear staining is present in the more centrally located superficial epithelial cells *(arrows)*.

intraepithelial neoplasia may identify a group epidemiologically distinct from other invasive cancers.

In this study, we analyzed a series of vulvar resections from 42 women with squamous cell carcinoma of the vulva. Our goal was to determine the frequency of detection of HPV nucleic acids and whether this parameter could be related to age, morphologic growth pattern, smoking history, and clinical behavior. We show that HPV nucleic acids are preferentially associated with a specific morphologic subtype of vulvar carcinoma and that this subtype is significantly associated with a younger age population and smoking history vis-a-vis other squamous cell carcinomas of the vulva.

Material and methods

Hypotheses to be tested. We intended to determine the proportion of conventional invasive cancers that were not associated with HPV nucleic acids and whether a subset of such patients would be distinguished by virtue of one or more morphologic, clinical, or behavioral parameters from the patients whose lesions were associated with HPV.

Patient selection and clinical data. From January 1978 to December 1987 the pathologic records of all women evaluated and treated for vulvar squamous cell carcinoma at the University of Virginia Health Sciences Center were reviewed. Retrospective chart review was performed and pertinent clinical data abstracted and entered for statistical analysis. Data obtained included age, smoking history, clinical stage of carcinoma, and survival. All histologic material was retrieved where possible, and cases with paraffin-embedded tissue were selected.

Histologic analysis. Cases were segregated into three morphologic categories: (1) Well-differentiated keratinizing (11 cases), (2) moderately and poorly differentiated (19 cases), and (3) intraepithelial-like (12 cases) squamous cell carcinoma. The latter contained areas of invasive neoplasm with a growth pattern similar to that of intraepithelial neoplasia (i.e., the cells were arranged in concentric nesting patterns with peripheral palisaded nuclei; Figs. 1 and 2).

Analysis for HPV nucleic acids. Serial sections from each tumor were analyzed for HPV nucleic acids by DNA-DNA in situ hybridization with biotinylated probes (for HPV types 6/11, 16/18, 31/33/35; VIRATYPE, Life Technologies, Gaithersburg, Md.). Because the goal was to determine whether HPV nucleic acids were present rather than determine specific type, hybridizations were carried out under conditions similar to those recommended by the manufacturer, with posthybridization washes performed at 42° C. Under these conditions, HPV type 16/18 DNA probes produced positive hybridization signals with formalin-fixed paraffin-embedded HeLa cells, which contain 10 to 20 copies of HPV type 18 DNA.

Statistical analysis. Statistical analysis was performed with a p value of 0.05 or less for statistical significance. Ordinal data were analyzed with comparison of median values with the Mann-Whitney U test; ratio data were analyzed with the Student t test; nominal data were analyzed with either χ^2 or Fisher's exact test. Kaplan-

Table I. Clinical characteristics correlated with histologic type

	Squamous cell carcinoma			
	IL	*WDK*	*MP*	*Total*
Age (yr)				
Mean	63.5	72.3	70.8	69.4
Median	64	73	72	70
Range	44-82	48-86	53-90	44-90
Gravidity				
Median	2	3	2	2
Range	0-8	1-9	0-7	0-9
Race				
White	8	10	18	36
Black	4	1	1	6
Smoking				
Yes	7	2	3	12
No	1	6	7	14
Not known	4	3	9	16

IL, Intraepithelial-like squamous cell carcinoma; *WDK,* well-differentitated keratinizing squamous cell carcinoma; *MP,* moderately to poorly differentiated squamous cell carcinoma.

Meier actuarial survival curves were compared with the Z scores.

Results

Clinical data. Forty-two cases were selected on the basis of the availability of material for histologic and viral analysis. Thirty-six were white and six black, and the ages ranged from 44 to 90 years with a mean of 69.1 ± 11.3 years and a median of 70 years. Gravidity ranged from 0 to 9 with a median of 3 (Table I).

From chart review it could be determined that 12 of the 42 women had a history of current or past tobacco use, with 14 known definitely to have never smoked. The smoking history of the remaining 16 patients could not be determined (Table I).

Tumor stage at initial presentation was as follows: International Federation of Gynecology and Obstetrics classification (FIGO) stage I, eight patients; stage II, 13 patients; stage III, 15 patients; and stage IV, six patients. Thirty-two women were treated by surgery alone, three by a combination of surgery and radiation, and the remaining seven by radiation alone (Table II).

Patients treated by surgery alone included six with radical vulvectomy, two with radical vulvectomy and unilateral groin node dissection, 22 with radical vulvectomy and bilateral groin node dissection, one with radical vulvectomy and abdominoperineal resection of the rectum and perineum, and one with radical vulvectomy and abdominoperineal resection of the rectum and perineum and unilateral groin node dissection (Table II).

Of the 28 patients with either unilateral or bilateral groin node dissections (with or without irradiation), 17 were found to have negative lymph nodes, seven had positive ipsilateral groin nodes, three had positive bilateral groin nodes, and one had a positive retroperitoneal (Cloquet's) node.

The adjusted actuarial survival rate for the 42 patients at 5 years was 81.4% (±6.4%). The FIGO stage I patients had an adjusted actuarial survival rate of 100% at 5 years, with 91.7% (±7.9%), 72.2% (±11.8%), and 60.0% (±21.9%), adjusted actuarial survival rates for stages II, III, and IV, respectively (Fig. 3).

Histologic analysis. Of the 42 cases, 11 were well-differentiated keratinized tumors, 19 were moderately to poorly differentiated, and 12 were intraepithelial-like. Coexisting vulvar intraepithelial neoplasia was found in none, none, and 33% of the above groups, respectively. Clinical features that correlate with these subtypes are summarized in Table I. Patients in the intraepithelial-like subgroup were significantly younger ($p = 0.04$) than the other groups. In addition, a significantly higher proportion of the intraepithelial-like subgroup reported a history of smoking than that of the other two groups ($p = 0.01$). Race and gravidity were not associated significantly with histologic subtype.

HPV DNA analysis. Twelve of 42 cases were positive for the presence of HPV DNA. Although the precise HPV type could not be determined because of the low stringency of hybridization, the strongest signals in each case were obtained with the HPV type 16/18 or HPV type 31/33/35 probes or both. The association of HPV DNA with histologic subtype is summarized in Table III and illustrated in Fig. 2. The intraepithelial-like carcinomas were significantly associated with HPV DNA ($p = 0.002$) vis-a-vis the other types. The likelihood of a lesion being HPV-positive was also associated with higher gravidity ($p = 0.05$) but not with a younger age ($p = 0.2$).

There was no correlation between survival and HPV DNA positivity. There was, however, bias in the groups because there was a statistically significant unequal distribution of treatment types between the HPV-positive and HPV-negative patients. Ninety percent (27/30) of the HPV-negative cases were treated by surgery alone in contrast with 6 of 12 (50%) HPV-positive cases that were treated with either radiation and surgery or radiation alone ($p = 0.01$). This bias was also reflected in an unequal distribution of treatment types as associated with histologic subgroup. Although 26 of 30 (86%) of the cases of well-differentiated keratinizing or moderately to poorly differentiated tumors were treated with surgery alone, only 6 of 12 (50%) of the intra-

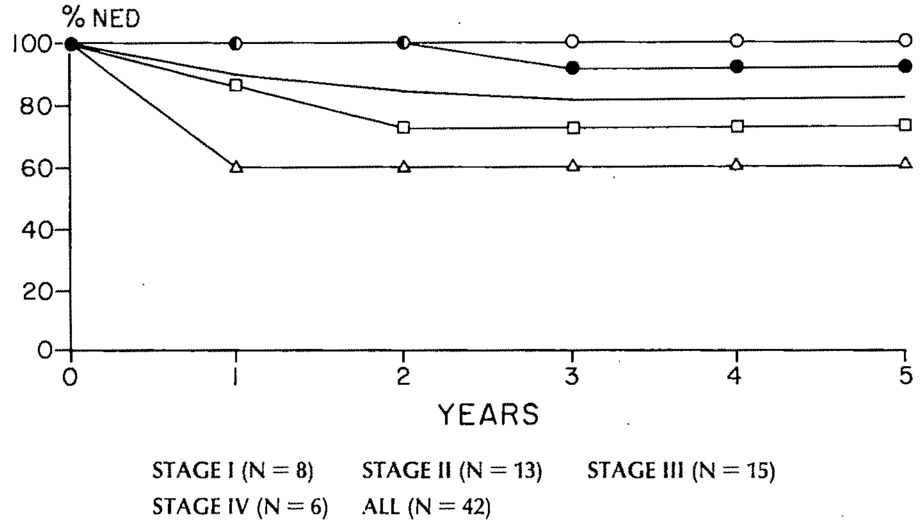

Fig. 3. Actuarial adjusted survival squamous cancer of the vulva, University of Virginia Health Sciences Center.

Table II. Clinical correlation stage versus treatment

Treatment	*Stage* I	*Stage* II	*Stage* III	*Stage* IV	Total
Radical vulvectomy	2	4	—	—	6
Radical vulvectomy, unilateral groin node dissection	2	—	—	—	2
Radical vulvectomy, bilateral groin node dissection	4	9	9	—	22
Abdominoperineal resection of rectum, radical vulvectomy	—	—	—	1	1
Abdominoperineal resection of rectum, radical vulvectomy, unilateral groin node dissection	—	—	—	1	1
External beam irradiation therapy and radical vulvectomy, bilateral groin node dissection	—	—	1	1	2
Radical vulvectomy, node biopsy, external beam irradiation therapy	—	—	—	1	1
Radiation therapy only	—	—	5	2	7
TOTAL	8	13	15	6	42

epithelial-like group were managed exclusively by surgery.

Comment

Although it has been assumed that a large proportion of invasive cervical carcinomas evolve from intraepithelial precursor lesions, the data in support of this mechanism for the development of vulvar cancer have been conflicting. Most compelling has been the marked disparity in the mean ages of women with vulvar intraepithelial neoplasia versus invasive cancer.[1-3] Moreover, several studies have reported that the majority of vulvar carcinomas are not associated temporally or topographically with typical precursor lesions such as vulvar intraepithelial neoplasia, but with hyperplastic or inflammatory epithelial changes (dystrophies) with various grades of cytologic atypia.[8, 10]

Because papillomaviruses and sexual factors have been closely associated with vulvar intraepithelial neoplasia, attention has naturally focused on the relationship between these factors and invasive cancer. However, both sexual and nonsexual causes have been proposed. For example, Mabuchi et al.[9] observed little relationship between invasive vulvar carcinoma and a history of condylomata. In contrast, these authors[9] found a relationship between vulvar carcinoma and occupational exposures, caffeine consumption, and smoking in younger women. A different perspective

Table III. Correlation of histology with clinical and viral data

			Ever smoked		
Histologic group	*No.*	*Mean age (yr)*	*No.*	*%*	*HPV+ (%)*
WDK	11	73	2/8	25	9
MP	19	72	3/10	30	16
IL	12	64*	7/8	88*	67*

WDK, Well-differentiated keratinizing squamous cell carcinoma; *MP*, moderately to poorly differentiated squamous cell carcinoma; *IL*, intraepithelial-like squamous cell carcinoma; *HPV+*, human papilloma virus–DNA positive.

*Significant ($p < 0.05$, Student's t test).

was put forth by Brinton et al.[11] who reported a powerful association between a history of condylomata or smoking and both vulvar intraepithelial neoplasia and invasive vulvar carcinoma. These authors[11] concluded that sexually transmitted agents occupied a central place in the pathogenesis of vulvar cancer.

A simple resolution of the disparity between the conclusions provided by the studies described here is problematic. However, it is noteworthy that the population studied by Mabuchi et al.[9] included a higher proportion of women older than 55 years of age than that of Brinton et al.[11] Thus it is likely that Mabuchi et al.[9] studied a population of which a larger proportion did not have associated intraepithelial disease. Indirect support for this is the much stronger association of vulvar neoplasia with smoking by Brinton et al.[11] in that this risk factor has been associated both with vulvar intraepithelial neoplasia and a younger age population.

The association between papillomaviruses and vulvar neoplasia has likewise been inconsistent and controversial. Although HPV type 16 nucleic acids have been strongly associated with intraepithelial neoplasia in several studies, the association between HPV nucleic acids and invasive carcinoma is weak.[7,15,17] Twiggs[17] observed that only 17% of invasive vulvar carcinomas were positive by in situ hybridization, similar to the results of this study. A possible link between HPV positivity and smoking in vulvar carcinoma has been observed.[15] Combined with the strong association between smoking and vulvar intraepithelial neoplasia, this suggests that one population of vulvar cancers may be identified by a strong smoking history, coexisting vulvar precursors, a younger mean age, and the presence of papillomavirus nucleic acids, implying in turn the role of sexual factors. This study supported this with the additional observation that most vulvar carcinomas associated with HPV nucleic acids were recognizable by the presence of certain morphologic features that resembled intraepithelial disease, even when a coexisting precursor (VIN) lesion was not identified.

In contrast, evidence has begun to accumulate that cancers that are not associated with vulvar intraepithelial neoplasia, and specifically those associated with vulvar inflammatory conditions such as lichen sclerosis, are infrequently associated with HPV nucleic acids.[16] Combined, these data strongly suggest at least two different pathogenetic mechanisms for vulvar squamous cell carcinoma.

It remains to be confirmed whether pathologic analysis will segregate the majority of HPV-related vulvar neoplasms from those that are not. However, the observations in this report combined with the negative association between coexisting lichen sclerosis and HPV-related carcinomas support the use of both viral and histologic parameters in epidemiologic studies of vulvar carcinomas. Such studies may be instrumental in placing the role of viral sexual factors in proper perspective and in targeting other risk factors associated with a large proportion of vulvar carcinomas in older women.

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Discussion

DR. FRED T. GIVEN, JR., Norfolk, Virginia. Dr. Andersen et al. address the association of the HPV and invasive squamous cell carcinoma of the vulva and correlate this with age, smoking history, and morphology. For my comments I am indebted to the expertise of two of my colleagues at the Eastern Virginia Medical School, Dr. Ken Somers, Professor of Microbiology and Immunology, and Dr. Jeng G. Hsiu, Associate Professor of Pathology. The primary goal of the authors was to determine by the use of in situ hybridization the frequency of association of the HPV and the correlation of this occurrence with specific morphologic types of vulvar cancer as well as age and smoking history. The evidence presented is interpreted to show that HPV DNA is preferentially associated with a specific morphologic subtype of vulvar carcinoma (intraepithelial-like lesions) and that the intraepithelial-like subtype is associated with a younger mean age and a positive smoking history. The results of this study are only as good as the sensitivity of the assay for the HPV DNA. The in situ hybridization assay used to detect HPV DNA in this article is the only method that allows precise microscopic detection of HPV nucleic acids within cells. The major drawback of the in situ test is its reduced sensitivity, relative to Southern blot or dot blot hybridization in the detection of latently HPV-infected tumors. Latently infected tumors or adjacent normal epithelium would likely receive a negative score in the in situ assay. Therefore the absence of HPV DNA in vulvar squamous cell carcinoma does not exclude an HPV etiologic factor. It is also important to note that negative HPV DNA results only mean that the test is negative for the HPV types evaluated.

The morphologic interpretation and proposed histologic criteria of intraepithelial-like invasive squamous cell carcinoma of the vulva in this article includes nests of invasive neoplastic epithelium with preserved cell polarity, similar to intraepithelial disease. This definition seems to create some confusion and uncertainty for the following reasons: (1) Vulvar intraepithelial neoplasia represents a continuous morphologic spectrum of epithelial cellular abnormality and maturation. Vulvar intraepithelial neoplasia is divided into types I, II, and III. Do the authors mean the intraepithelial lesion mimics vulvar intraepithelial neoplasia type III? It would seem difficult to classify the intraepithelial-like invasive squamous cell carcinoma into a separate distinct morphologic entity on the basis of their proposed criteria. (2) It is also known that invasive squamous cell carcinoma of the vulva is composed of a heterogeneous morphologic pattern of neoplasia, even within the same tumor. Focal areas of vulvar invasive squamous cell carcinoma may fit into the author's criteria of intraepithelial-like growth pattern, but other areas of the same

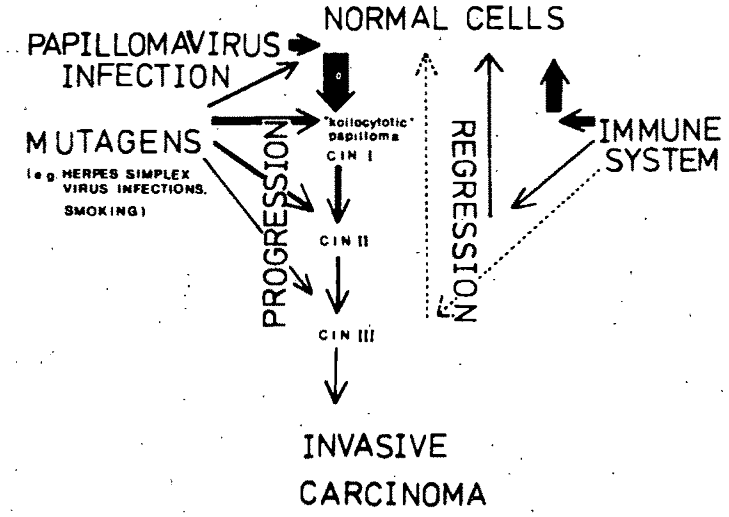

Fig. 1. Proposed model for synergistic effects in human genital cancer. (From zur Hausen H. Lancet 1982;2:1371. By permission.)

tumor may show a totally different morphologic growth pattern. How will the authors clarify such squamous cell carcinomas that comprise different patterns?

The data on smoking cause me some concern and that concern is confounding. This was well pointed out in an editorial.[1] There may be many other initiating events or promoting factors in the cause of vulvar cancer, just as there are in carcinoma of the cervix. This is certainly well illustrated in the model proposed by Dr. zur Hausen (Fig. 1).[2]

The author's data certainly seem to show some association between the papillomavirus and intraepithelial-like carcinoma of the vulva. This seems to occur at a somewhat younger age; however, it remains to be seen whether survival is affected by being able to isolate this particular subgroup of patients. Is the biologic behavior of the intraepithelial-like lesion described by the authors different? This study should be repeated with an even more sensitive technique, specifically, polymerase chain reaction amplification of HPV DNA with primers that recognize highly conserved regions within the HPV genome.[3] Until these data are presented, negative results should be interpreted with caution. There should also be adjustment for confounding, such as the sexual activity, number of partners, exposure to passive smoke and caffeine if they want to truly see the effects of smoking per se on the epidemiology of carcinoma of the vulva. We believe this study should be continued in much more detail and with a larger number of patients, perhaps requiring a multiinstitutional protocol to confirm these preliminary results.

I have three questions for Dr. Andersen:

1. Do you believe that you will be able to classify the intraepithelial-like invasive squamous cell carcinoma of the vulva in a heterogenous type of neoplasm?

2. Do you believe that with a much larger series the group with the intraepithelial-like growth pattern will have a different survival rate than other invasive squamous cell carcinomas of the vulva?

3. Are you convinced of the significance of the smoking data with a series this small?

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Dr. Matthew O. Burrell, Atlanta, Georgia. Did the authors have any cases with preexisting or coexisting lichen sclerosis? In Atlanta we have had four patients in whom early invasive carcinoma has developed in preexisting lichen sclerosis.

Dr. Andersen (Closing). We looked at the surrounding epithelium in most cases and four of 12 with an intraepithelial-like pattern had associated vulvar intraepithelial neoplasia. Eight of the remaining 28 were associated with some other form of vulva dystrophy. We had one of the 42 in whom an invasive cancer developed while being folowed up for vulvar dystrophy. Therefore I think vulva dystrophies demand careful attention and follow-up.

To address Dr. Given's comments and concerns, it is Dr. Crum's belief that the intraepithelial-like pattern can be distinguished by the orientation of the cells in circumscribed nests with retention of cell polarity and palisaded nuclei at the periphery (Figs. 1, *C*, and 2, *A*). This arrangement of cells is similar to that seen in vulvar intraepithelial neoplasms, irrespective of grade, but cannot be compared directly with them gradewise because this pattern is being described in invasive carcinomas. In all the intraepithelial-like lesions, this growth pattern was clearly present in an invasive component by virtue of the stromal reaction around the nests (Fig. 1, *C*). We agree that lesions may be heterogeneous and contain more than one pattern, but the intraepithelial-like pattern was classified separately because of its distinctive appearance. The association of HPV nucleic acids with intraepithelial-like neoplasms may be fortuitous or reflect the fact that invasive carcinomas within this growth pattern are more likely to have derived from vulvar intraepithelial neoplasms. As you know, a high percentage of the latter are known to contain genital papillomavirus nucleic acids.

Whereas a variety of other techniques have been used to search for HPV DNA in human cells, we believed in this retrospective study that the in situ hybridization technique was the most appropriate because it provided for direct localization of HPV nucleic acids in the cells of the neoplasm. The polymerase chain reaction, although superior in sensitivity, does not establish the location of the viral nucleic acids detected and can be plagued by false-positive results. I would reemphasize that the hybridization conditions used by Dr. Crum permitted detection of not only HPV DNAs identical to those in the probes, but more distantly related HPV types as well, and the sensitivity was such that as few as 10 to 20 copies of HPV DNA could be detected in an individual cell.

Our smoking data were based purely on chart review and in many instances we could not tell whether the patients had ever smoked. The issue of smoking exposure is quite complex and although in our small series with small numbers we can show a statistical association between smoking and intraepithelial-like histologic pattern as well as the presence of HPV, clearly this is only a preliminary observation that requires much more sophisticated epidemiologic study to confirm this potential association.

Treatment failure of genital condylomata acuminata in women: role of the male sexual partner

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Richmond and Falls Church, Virginia

The male sexual partners of women with genital condylomata acuminata are thought to be carriers of human papillomaviruses. It is therefore often recommended that both sexual partners be treated. We studied 360 women with genital warts to test the hypothesis that when the male partners of women with condylomata acuminata are treated, the treatment failure rate decreases. The male sexual partners of 180 of these women were examined and, if indicated, treated (treatment group). The 180 remaining male sexual partners were neither examined nor treated (control group). One hundred twenty-two (68%) men in the treatment group had human papillomavirus–associated lesions. The treatment failure rate of women was 16.7% in the treatment group and 18.9% in the control group. The difference is not statistically significant ($p > 0.05$). The results of this study do not support the hypothesis that the treatment failure rate of women with condylomata acuminata decreases if their male sexual partners are also treated. (AM J OBSTET GYNECOL 1991;165:337-40.)

Key words: Condylomata acuminata, human papillomavirus infection, human papillomavirus in men, treatment failure of condylomas

Genital condylomata acuminata are sexually transmitted and become clinically apparent 3 weeks to 8 months (average 3 months) after infection.[1] Although 65% of men have genital lesions associated with human papillomavirus (HPV) when condylomas are found on their female partners, many affected men have no symptoms and are not aware of their lesions.[2] Because penile condylomata acuminata are thought to be contagious, many physicians advocate treatment of the infected male partner to prevent reinfection of the female partner and treatment failure.[1] There is however only anecdotal evidence to suggest that women treated for condylomata acuminata have recurrences unless their male sexual partners are also treated.[3]

In this report we test the hypothesis that the control rate of genital condylomata acuminata in women is improved if their male sexual partners are also treated.

Material and methods

The study was carried out retrospectively; it included 360 women whom we examined and treated for genital condylomata acuminata. The male partners of 180 women were examined and biopsies were performed; if lesions were documented (treatment group), the men were treated. The women were matched with 180 comparable women (Table I) whose male sexual partners were neither examined nor treated (control group). Each patient claimed to have maintained a monogamous sexual relationship during the time of treatment and follow-up. Women in both groups were advised to abstain from sexual intercourse or to have their partners use condoms until follow-up examinations showed no evidence of condylomata acuminata.

Follow-up examinations of all men and women enrolled in the study included colposcopy and were carried out every 1 to 3 months for at least 1 year. The duration of follow-up for men was 12 to 29 months (mean 14.8 months); for women it was 12 to 59 months (mean 22.3 months). All treated men had their lesions eradicated with treatments previously described.[4]

All women enrolled in the study responded to therapy and were found to be without evidence of condylomas for at least 1 month during follow-up. Therefore all of them were permitted to resume sexual intercourse without condom protection. For this study treatment failure is defined as a reappearance of condylomata acuminata in a patient previously documented to be free of visible condylomata acuminata for at least 4 weeks after completion of therapy. Treatment failures detected within 6 months of completion of therapy were regarded as being early. Treatment failures detected after 6 months were regarded as being late.

Results

One hundred twenty-two (68%) of the 180 men examined had HPV-associated lesions. Fifty-six men

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Table I. Comparison of study groups

	Partner treated (n = 180)	*Partner untreated (n = 180)*	*p*
Age (yr)			
Range	17-58	15-47	
Mean	28.4	26.9	>0.05*
Race (%)			
White	56	49	>0.05†
Black	41	46	
Other	3	5	
Status (%)			
Private patient	78	72	>0.05‡
Nonprivate patient	22	28	
Immunosuppression	0	0	
Smoker (%)	23	26	>0.05‡
Oral contraceptives (%)	39	44	>0.05‡
Pregnant	0	0	
Extent of condylomas (%)			
Mild§	14	17	>0.05†
Moderate‖	43	44	
Severe¶	43	39	
Organs involved (%)			
Vulva only	32	35	>0.05†
Vagina only	14	16	
Multiple organs#	54	49	
Therapy (%)			
Topical only**	22	27	>0.05†
Surgical only††	15	14	
Several	63	59	

*Student's *t* test.

†Test of homogeneity.

‡χ^2 test.

§<5 Lesions or <5 cm^2.

‖5 to 15 Lesions or 5 to 10 cm^2.

¶>15 Lesions or >10 cm^2.

#Including urethra and anus.

**5% 5-Fluorouracil, trichloroacetic acid, podophyllin.

††Laser vaporization, cryotherapy, electrocautery, excision.

(46%) had condylomata acuminata; the others had papular, papillary, spiked, or flat lesions. Forty-two (34%) of the epithelial abnormalities were subclinical and could only be seen under magnification, with or without application of acetic acid. The lesions were mostly on the prepuce (71%) or penile shaft or both (54%). Lesions were less commonly found on the glans (9%), scrotum (5%), urethral meatus (5%), urethra (3%), and other areas, including thigh and anus (7%). The number of lesions ranged from 1 to >100 (median 5) and measured from <1 to 55 mm (median 3 mm) in diameter.

The time from the initiation of therapy to control of the women's lesions ranged from 1 to 272 days (mean 77.3 days) in the treatment group and from 1 to 385 days (mean 96.8 days) in the control group. The difference was not statistically significant (Student's *t* test, $p > 0.05$).

Treatment failed in 30 (16.7%) of the 180 women in the treatment group and in 34 (18.9%) of the 180 women in the control group. The difference in the treatment failure rate between the two groups was not statistically significant ($p > 0.05$, χ^2 test).

Twenty-five (83.3%) of the 30 treatment failures in the treatment group were early; five (16.7%) were late. In the control group 25 (73.5%) of the 34 treatment failures occurred early and nine (26.5%) occurred late. The difference in early and late treatment failures between the two groups of patients was not statistically significant (χ^2 test, $p > 0.05$).

Comment

In a previous study we showed that the treatment of genital condylomas in men does not decrease the treatment failure rate of cervical intraepithelial dysplasia in the female sexual partner.[5] The current study was carried out in a similar fashion in a different patient population to investigate whether examination and treatment of the male sexual partner lowers the treatment failure rate of condylomata acuminata in women. It became apparent that neither the incidence nor the time of recurrence of condylomata acuminata in women changed significantly when their male sexual partners were also examined and treated. The figures suggest that reinfection by the same male sexual partner is not an important cause of treatment failure of condylomata acuminata in women.

Reasons for treatment failure may be found in the natural history and pathophysiology of the infection. It is recognized that HPV infections involve the entire genital tract.[6,7] By contrast, manifestation of the condylomas is most often focal rather than diffuse and is probably influenced by the part of the genital tract involved, as well as by immunologic and virologic factors. The importance of cellular immunity in suppressing HPV infections is suggested by studies in persons with genetic, induced, or acquired cell-mediated immunodeficiencies. It has also been found that a subset of intraepithelial Langerhans cells, which are considered to be the first line of host defense, are locally depleted in women with HPV infections.[8] These factors may be of greater importance than reinfection with an HPV strain that patients continue to harbor in latent or subclinical form regardless of treatment attempts.[7,9] Hence, neither the treatment of the same male partner nor the use of condoms can protect from reactivation of preexisting infection. Nonetheless it is theoretically possible for a man with condylomas to pass such a high viral load to his female partner during coitus that the relative immunity of the woman with latent infection is overwhelmed and new condylomas develop. This most likely occurs when condylomata acuminata are present because these lesions contain the highest number of viruses per cell and shed the largest number of cells per contact compared with noncondylomatous lesions.[10, 11] Therefore, besides preventing HPV-associated problems in future sexual partners, treatment of exophytic condylomas in men may also be helpful to their current women partners.

It is possible that the design of the study did not permit detection of a significant difference between the two groups of patients studied. From examination of the male partners in the treatment group, it could be anticipated that approximately two thirds of the male sexual partners in the control group had HPV-associated lesions. That incidence is similar to the incidence of condylomas in men reported by other authors.[2, 12] On the basis of the findings in the treatment group, it may also be expected half of the HPV–associated genital lesions of the untreated men to be condylomata acuminata. However, many of these lesions are grossly visible and some men with condylomata acuminata may have sought medical attention, leaving relatively fewer men with exophytic lesions in the control group as compared with the treatment group. Therefore men in the control group may have been relatively less infectious than men in the treatment group.

A more perfect match of male partners could be made if all of the men were examined but only half were treated. This study design, however, would be ethically objectionable.

Another problem with the design of our study may be the recommendation that women have their male partners use condoms during intercourse as long as condylomas are present. Although all women claimed to have resumed sexual intercourse without condom protection after they were found to be free of condylomata acuminata, there was no accurate information as to the frequency of unprotected intercourse. High coital frequency with an infected person has been found to be a risk factor for HPV infection.[10]

In spite of these methodological shortcomings, this study does not support the commonly held belief that the treatment failure rate of genital condylomata acuminata in women may be lowered if the male sexual partner is also treated.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. WESLEY C. FOWLER, JR., Chapel Hill, North Carolina. HPV and its related disorders, including condylomas and intraepithelial neoplasia of the female genital tract, require more and more of the physician's time, not only in attempting to treat the disorder. Also the time it takes to counsel these patients and their partners is endless. Reasonable estimates show that 25% of the women in the United States harbor HPV. It seems more and more that we know less and less about the management of HPV.

Dr. Krebs has contributed much to our knowledge of HPV-related disorders. I consider him to be a pioneer in the use of 5-fluorouracil to treat genital HPV. He addresses a question that many of us have struggled with and don't have an answer for. Should we treat the male sexual partners of women who have condylomas?

This is a retrospective study of 360 women who were examined and treated by the authors for genital condylomata acuminata. In half the women the male partners were examined and treated; in the other half no examination or treatment of the male partners was begun. Dr. Krebs's results show that there was no difference in treatment failures in the women regardless of whether their male partners were treated. Treatment failed or disease recurred in 15% to 20% of the women.

Dr. Krebs and his associate should receive an award. What a tour de force the follow-up was! Patients obviously came back religiously for Dr. Krebs to be able to pick up treatment failures only 4 weeks after patients were declared HPV free. The patients had great faith in their physicians; all 360 women were monogamous and complied with requests to use condoms or abstain from intercourse while undergoing treatment. The authors are to be commended for so greatly influencing their patients' behavior.

I have several questions for Dr. Krebs:

1. How did he select the women? Were there any exclusions because of follow-up problems? How did he find 360 women who were monogamous?

2. Were biopsies performed for all of the men? If not, could some men who had been thought to have HPV lesions not have had them, that is, did they have skin tags or benign epithelial lesions?

3. How many patients in his current study were also in his previous study?[1] How does he account for the difference in the colposcopic findings in the men in the two studies? In the 1990 study the men had more papules, and 50% required colposcopic identification. In this presentation 50% of the men had condylomas and only one third required colposcopic visualization.

4. Finally, can we say that HPV-related lesions are now so prevalent that we can no longer consider HPV to be a sexually transmitted disease?

I appreciate Dr. Krebs's sharing this information with us. I am sure these data will aid us in the management of our patients' HPV.

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Dr. Krebs (Closing). In retrospective studies it is difficult to ascertain all factors, such as condom use during intercourse, sexual abstinence, and the involvement of other sexual partners, with reasonable accuracy. This information is difficult to obtain even in prospective studies. All data in the present study were obtained from patients visiting the clinic or the private office. Although many more clinic patients than private patients were treated in the past, the present study is heavily weighted in favor of private patients because of follow-up problems with clinic patients.

The high incidence of condylomata acuminata as opposed to papular or flat lesions in the male partners in this series is because the women had exophytic lesions, which are associated with HPV types 6 and 11. These HPV types also cause condylomata acuminata in men. In our previous study, we reported on women with cervical intraepithelial neoplasia. The corresponding genital lesions of this type in men are usually papular or flat because of association with HPV types other than 6 and 11.

As to the high prevalence of HPV, the question was raised as to whether HPV-associated changes in the genital area can still be regarded as a sexually transmitted disease. Although HPV has been found in approximately 25% of women who had no symptoms, the figure would probably be much higher if more sensitive probes were used for HPV detection. By using polymerase chain reactions, researchers have found HPV in 80% to 90% of sexually active women. Those figures, however, have been disputed and await further study. If they are confirmed, we need to reconsider our theories that HPV is related to genital cancer and should consider HPV to be merely a saprophyte.

Partial dissection of the zona pellucida of frozen-thawed human embryos may enhance blastocyst hatching, implantation, and pregnancy rates

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The rate of successful implantation after replacement of frozen-thawed embryos in in vitro fertilization is commonly only 5% to 10% per embryo. A limiting factor may be inability of otherwise viable embryos to be released from the intact zonae pellucidae. Culture conditions and/or cryopreservation in in vitro fertilization may affect the zona and impair blastocyst hatching. Therefore opening of the zona by partial slicing by means of micromanipulation before replacement of early cleaved embryos may improve chances of eventual hatching (referred to as assisted hatching). In 65 thawed embryo replacement cycles methyl-prednisolone and antibiotics were given for 4 days mid cycle. Assisted hatching was performed in 32 cycles, with 33 cycles left as controls. Patient's age, infertility, cycle supplementation, and number of thawed and replaced embryos did not differ significantly between the two groups. Rates of viable embryonic implantation were 16% (10/63) and 9% (6/64) in the assisted hatching and control groups, respectively. Group sizes need approximately to double before this trend toward improved implantation with the use of assisted hatching reaches statistical significance. (AM J OBSTET GYNECOL 1991;165: 341-5.)

Key words: Zona pellucida, micromanipulation, cryopreservation, blastocyst hatching

The human zona pellucida, a relatively simple glycoprotein structure surrounding both the unfertilized oocyte and the early embryo, has a surprising number of biologic roles. Either these are temporally brief, such as the species-specific binding of spermatozoa to the oocyte and the subsequent block to polyspermy, or they exist over longer periods, principally as a mechanical barrier to prevent disruption of the oocyte or embryo within from immune cell attack, physical squeezing, or temporary biochemical toxicity.

Recently, certain new techniques in human in vitro fertilization and embryo transfer have highlighted the major functional aspects of the zona pellucida. Techniques to increase chances of fertilization have necessarily focused on broaching the zona pellucida to assist passage of spermatozoa[1] to or placement of spermatozoa[2] next to the oolemma. Blastomere biopsy at the embryo cleavage stage for genetic analysis[3] introduces a hole in the zona pellucida after fertilization. All of the embryos from these studies therefore have incisions in the zonae pellucidae but nonetheless have led to live births. Cohen et al.[4] have proposed that a hole in the zona pellucida may be of benefit, because it may enhance blastocyst hatching, implying that some proportion of in vitro fertilization–generated embryos actually may fail to implant for no other reason than that they are unable to shed the zonae pellucidae.

An initial study in which fresh, 2-day-old, cleavage-stage embryos underwent partial dissection of the zona pellucida before uterine transfer[5] strongly suggested that this hole in the zona improved chances of implantation. This technique (referred to as assisted hatching) has subsequently been used with cryopreserved embryos after thawing to determine if chances of implantation can be improved in such cases.

Material and methods

Sixty-five patients who had cryopreserved embryos that survived thawing before uterine replacement were considered for this study. Thirty-three patients were allocated on an alternating basis to the control group (embryos left with intact zonae pellucidae), and 32 were assigned to the experimental assisted hatching group. Embryos were originally generated from ovarian stimulation protocols, as previously described,[6] with the majority in both the control and assisted hatching groups from cycles stimulated with a combination of clomiphene citrate (Serophene; Serono, Norwell, Mass.) and human menopausal gonadotropin (Pergonal, Serono).

All embryos had been cryopreserved at between the

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6/6/30073

Table I. Patient data in zona-dissected and control groups

	Status of zona pellucida	
	Intact (control)	*Incision (assisted hatching)*
Patients		
Total No.	33	32
Age (yr)	36.0 ± 3.7*	33.9 ± 3.9
Principal type of infertility		
Tubal	11	11
Endometriosis (mild to moderate)	5	7
Male factor	4	3
Adhesions	3	2
Ovulatory dysfunction	3	5
Idiopathic	3	2
Diethylstilbestrol exposure–cervical stenosis	2	1
Immune (male and female)	2	1

*Mean ± SD.

Table II. Embryonic survival after thawing and number of embryos subsequently replaced in zona-dissected and control groups

	Status of zona pellucida	
Embryo	*Intact (control)*	*Incision (assisted hatching)*
Thawed (No.)	2.7 ± 1.0*	2.6 ± 0.8
Pronucleate (%)	41.7	49.4
Partial survival† (%)	30.7	25
Survived and replaced (No.)	2.1 ± 0.7	2.0 ± 0.8

*Mean ± SD.

†Percentage of cleavage-stage embryos in which not all blastomeres survived the thaw (e.g., a four-cell embryo surviving as two of four cells).

zygote (one-cell) stage and the six-cell stage in 1.5 mol/L 1,2-propanediol and 0.1 mol/L sucrose, and then they were stored in 0.75 ml glass ampules.[6] This process was relatively rapid, with embryos being taken directly from 37° C culture into 1.5 mol/L 1,2-propanediol at room temperature for 10 minutes; this was followed by transfer into 1,2-propanediol plus 0.1 mol/L sucrose. Embryos were then immediately placed individually into indelibly marked glass ampules that were heat sealed before introduction into a programmable liquid nitrogen freezer. From room temperature embryos were cooled at a rate of −2° C/min to −6.5° C, at which temperature they were held for 15 minutes. After 5 minutes' holding time, the ampules were manually seeded with chilled forceps. Finally, embryos were cooled at a rate of −0.3° C/min to −36° C and plunged into liquid nitrogen ready for storage. Thawing was performed rapidly in a 31° C water bath, followed by washing at room temperature through a series of 1,2-propanediol dilutions in 0.2 mol/L sucrose. A final six washes were carried out in fresh culture medium before culture at 37° C. Thawed zygotes were cultured for up to 24 hours to observe cleavage, and cleavage-stage embryos, when thawed, were left for a minimum of 2 hours in culture to check viability. All culture was carried out in sodium bicarbonate–buffered Earle's balanced salt solution, supplemented with 15% maternal serum or from screened donor serum.

All patients received a combination of 16 mg methylprednisolone (Medrol, The Upjohn Co., Kalamazoo, Mich.) daily, plus 250 mg tetracycline (Barr Laboratories, Pamona, N.Y.) four times daily from the day of estimated ovulation for 4 days, to prevent possible leukocytic invasion of the early embryo through the hole in its zona pellucida. No harmful side effects arose in any of these patients or in previous studies where this medication had been used in this fashion.[7] All patients were ovulatory; however, in some cases 50 mg clomiphene citrate was administered daily from day 5 to day 9 to regularize ovulation[8] in 16 patients in the assisted hatching group and 20 in the control group. Ovulation was assessed in all cases by urinary luteinizing hormone (LH) assay (LH MAIAclone, Serono) to monitor the endogenous LH surge. Two patients in the control group and one in the assisted hatching group received an additional 3000 U human chorionic gonadotropin (hCG) (Profasi, Serono) to initiate ovulation, because no endogenous LH surge occurred. Thawing was scheduled according to embryonic age with necessary adjustments made to accommodate embryonic-endometrial asynchrony.[8] Essentially, mock oocyte collection was assessed as occurring 25 hours after an LH surge. The age of the embryo was then added; this was determined to be the time elapsed between original oocyte collection and time of freezing, regardless of the time of insemination, although >90% of inseminations occurred between 3 and 6 hours after oocyte retrieval. In the case of zygotes thawing was performed 6 to 9 hours in advance of embryonic-endometrial synchrony. For cleaved embryos thawing was performed even earlier at 9 to 12 hours before synchrony. The "early" thawing of embryos compensates for two factors: first, a marginal slowing of embryonic development in vitro can be accommodated; second, the thawed embryo also requires some time to recover metabolically.

Within 2 hours before replacement all embryos in

Table III. Outcome of pregnancy from replacement of thawed embryos with and without zona dissection

	Status of zona pellucida				
	Intact (control)		*Incision (assisted hatching)*		
Definition of pregnancy	*No.*	*%*	*No.*	*%*	*Fisher's exact test*
Biochemical* (per transfer)	6/33	18	12/32	37	$p = 0.148$
Clinical (fetal heart beat)					
Per transfer	5/33	15	9/32	28	$p = 0.234$
Per embryo replaced†	6/64	9	10/63	16	$p = 0.240$
Term (per transfer)	4/33	12	7/32	22	$p = 0.290$

*Delayed menses with transiently raised β-hCG level.

†Number of viable fetal sacs per number of replaced embryos.

the assisted hatching group underwent partial dissection of the zona pellucida with fine, pulled glass pipettes and needles that were mounted on an inverted microscope with Nomarski interference optics and equipped with micromanipulators and an electrically heated stage. This procedure was carried out in a warm glass well slide with the embryo in a droplet of medium under mineral oil. The technique is identical to that described for partial zona dissection of unfertilized oocytes,[9] except there was no need to remove cumulus cells with hyaluronidase, and the embryo was not exposed to sucrose to shrink it and so increase the perivitelline space. All embryo replacements were performed as atraumatically as possible with the Wallace embryo replacement catheter (H.G. Wallace, Colchester, United Kingdom). Initial assays for β-hCG were carried out at 12 to 14 days after embryo replacement, with repeats at 3 to 7 days if positive, until an ultrasonographic scan was performed at approximately 7 weeks of pregnancy.

Results

Ages of patients were not significantly different between the control and assisted hatching (Table I), and no infertility type predominated significantly between the groups.

Marginally more pronucleate (one-cell) embryos were frozen and thawed in the assisted hatching group (49.4% vs 41.7%), with more cleavage-stage embryos being frozen and thawed in the control group (Table II). This fact accounted for the slightly better partial embryonic survival rate in the assisted hatching group (25% vs 30.7%), because zygote thawing success is an all-or-nothing event. Consequently, overall survival in the assisted hatching group is fractionally less (76.9% vs 77.7%). Nevertheless, very similar numbers of embryos were both thawed and replaced in both groups.

The comparison of pregnancy outcomes in these frozen-thawed embryos indicates a definite trend toward better viable implantation after application of the assisted hatching procedure (Table III). Because of the limited number of patients available for this study, the results have yet to reach statistical significance.

Comment

As appears to be the case with fresh embryos, a relatively small incision in the zona pellucida is not incompatible with development and implantation of frozen-thawed human embryos. In fact, these preliminary results even suggest that such a hole in the zona pellucida may improve chances of implantation, presumably through enhanced chances of hatching of the blastocyst. Malter and Cohen[10] showed that human and mouse embryos arising from oocytes fertilized by means of partial zona dissection hatched more frequently and at an earlier time than embryos with fully intact zonae. As an extension of this observation, it has been consistently noted that the viable embryonic implantation rate of embryos from fertilized oocytes after partial zona dissection in cases of male factor infertility is higher than that of conventionally fertilized oocytes.[5]

One of the few morphologic factors that significantly correlates with embryonic viability is the presence of thin patches in the zona pellucida of both fresh and frozen-thawed human embryos.[11] That this thinning of the zona is an active process, integral to the embryo itself, was noted by Wright et al.,[12] when the zonae of some 28% of in vitro cultured human zygotes were seen to become thinner during the period before uterine replacement at the cleavage stage. It is also possible that lysins within the female reproductive tract assist in the dissolution of the zona pellucida, a notion supported by the increased incidence of blastocyst hatching seen with human embryos cocultured with uterine cells.[13] However, this effect also could be due to the more expanded nature of cocultured embryos as they apply greater physical pressure to the surrounding zona pellucida.

It is known that in vitro culture causes "spontaneous" hardening of the zona pellucida in the mouse.[14] In the context of thawed embryo development, an additional factor may have some bearing on the benefit of assisted hatching for cryopreserved embryos. If we accept that there may be some change in the physical nature of the zona pellucida as a result of the cryopreservation procedure, a change that makes the zona more brittle and resilient to hatching, then dissection of the zona should ameliorate this to allow the blastocyst to release itself more easily at the time of hatching in utero. The zona of the unfertilized oocyte after cryopreservation is thought to differ markedly from that of the fresh oocyte, as seen in the lowered spermatozoal penetration of thawed oocytes.[15] Indeed, comparison of the mechanics of the zona dissection technique in both fresh and frozen-thawed embryos gives the impression that the fresh zona is considerably more malleable. Biochemical characterization of any such changes of the zona before and after cryopreservation needs to be established.

Cohen et al.[7] have discussed at length the means by which midcycle corticosteroids and antibiotics may protect zona-dissected preimplantation embryos. This work cited a small study of embryo transfer of partial zona dissection embryos, with and without corticosteroids and antibiotics, in which implantation was considerably improved in the group with midcycle supplementation. A more extensive study of the midcycle use of antibiotics and corticosteroids in a randomized fashion among all of our patients undergoing in vitro fertilization and embryo transfer has shown no intrinsic benefit for implantation with this supplementation regimen (unpublished observations). In this study all patients received this midcycle supplementation, and it is assumed that any enhancement of implantation is independent of the midcycle antibiotics and corticosteroids.

Assisted hatching has yet to prove significantly advantageous in the small group of patients so far considered. Numbers in this study need to be approximately doubled if the trend is to reach statistical significance. One obvious reservation of this study is that any extra handling of human embryos, in the form of micromanipulative zona dissection, may be potentially hazardous. However, while zona dissection proves not to be detrimental when compared with control, unmanipulated embryos, there seems every reason to continue this study to establish whether assisted hatching is truly beneficial to pregnancy outcome after embryo cryopreservation.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

DR. ANIBAL A. ACOSTA, Norfolk, Virginia. This article reviews the application of partial zona pellucida dissection to cryopreserved-thawed human preembryos and its effects on the implantation rate and pregnancy outcome. In general, the implantation rate is defined as the number of gestational sacs identified in relation to the number of preembryos transferred. The authors have considered only viable implantations (heartbeats present).

The changes induced to the zonae pellucidae of the preembryos after cryopreservation have not, to my knowledge, been elucidated. The very scanty information available refers to mouse oocytes cryopreserved at 4° C with a different procedure than the one used for human preembryo cryopreservation. A lower fertilization rate was observed,[1] probably because of induced zona reaction during either freezing or thawing. Zona drilling overcomes the problems, thereby indicating that the block of fertilization is at the level of the zona in the mouse system.[2]

There are clear indications in the literature that preembryos that are allowed to continue their development in vitro have a high degree of developmental arrest; only 30% reach the stage of expansive blastocyst and then hatch.[3] Therefore, at least under in vitro culture conditions, a significant amount of preembryos do not achieve a stage at which implantation can occur.

The field of assisted fertilization has introduced several different techniques of micromanipulation to try to overcome the problem of lack of fertilization. The results are so dismal, mainly if authentically poor-quality sperm is used, that its clinical usefulness in the foreseeable future is, to say the least, doubtful. Nevertheless, micromanipulation for the purpose of assisted hatching seems to have a more credible role in assisted reproduction.

Our implantation rate in Norfolk, Virginia, for fresh preembryos transferred for the years 1989 and 1990 was 11.2%.

The authors administered 50 mg clomiphene citrate to 16 of the 32 patients allocated to the assisted hatching group (50%) and to 20 of 33 patients in the control group (60.6%). Although in some studies[4] there seems to be no difference in the implantation rate of cryopreserved zygotes and preembryos when 50 mg clomiphene citrate was used, as in this investigation, a substantial part of controversial literature seems to indicate a possible deleterious effect of clomiphene citrate on endometrial morphologic characteristics, synchrony, development, receptor content, and pregnancy rates.[5-7] Therefore, in a study in which the end points are the preembryo implantation rate and pregnancy outcome, it will be safer, in terms of experimental design, to stay away from that treatment.

The number of preembryos cryopreserved and transferred in each one of the stages between zygote and six cells in the control and study group is not given, so we should assume that the distribution was even in both groups.

Other information that would be useful to have is the timing of the transfer during the natural cycle and the embryo survival rate after cryopreservation, which may be important to judge the embryo quality obtained with the protocol used for stimulation. Morphologic evaluation of the embryo quality in both groups would be another useful criterion.

The protocol for monitoring these patients and the time of ultrasonographic examination after a positive pregnancy test result would be other information of interest to the reader. Perhaps all these questions can be addressed by the authors.

The implantation rates, with only sacs with fetal heartbeats used as a reference point, are 9% and 16% in the control and study groups, respectively. As the authors stated, these numbers represent only a trend without actual statistical significance. The term-ongoing pregnancy rate per transfer in the control group is somewhat low by the present standards.

Just as a point of reference, the Norfolk preembryo cryopreservation program from April 1987 (time of inception) through December 1990 showed a 64.0% survival rate of both, out of the total number of preembryos thawed and out of the preovulatory oocyte–derived preembryos thawed. The implantation rate was 11.4% without artificial hatching.

I think this line of research should continue until we can demonstrate clearly whether zona hatching can improve the implantation and pregnancy rate of cryopreserved preembryos.

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Dr. Massey (Closing). The patients who received clomiphene were selected according to clinical criteria. Our survival of embryos is associated with about a 40% loss of embryos from the cryopreservation process. Our implantation rate per frozen embryo replaced is about 9%, as in the group that we reported. It is a good point that we should have made morphologic observations comparing the two groups of patients and relating them to survival and pregnancy rates. It is hoped that a year from now we will see real progress in this field.

Clinical Opinion

Polycystic ovary syndrome and the androgen-insulin connection

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The association of hyperandrogenism, insulin resistance, and polycystic ovarian disease is well established. The accompanying hyperinsulinemia results in acanthosis nigricans, an epiphenomenon of this syndrome. The knowledge that states of insulin resistance of diverse causes are associated with ovarian hyperandrogenism makes the argument for insulin-driven ovarian androgen secretion compelling. However, equally compelling evidence suggests that hyperandrogenism may contribute to insulin resistance and hyperinsulinemia. The irreconcilable differences between these two hypotheses have resulted in an array of contradictory studies. In this article a unified concept of polycystic ovary syndrome and its androgen-insulin connection is proposed. The hypothesis incorporates the role of hyperinsulinemia in the androgen excess observed (and vice versa); the key to this connection is the androgen-dependent change in regional body fat distribution and its metabolic consequence. The pathophysiologic features of polycystic ovary syndrome, which has important clinical sequelae, deserve further consideration. (AM J OBSTET GYNECOL 1991;165:346-8.)

Key words: Polycystic ovary syndrome, hyperandrogenism, insulin resistance, acanthosis nigricans

Since the original correlation of diabetes and hirsutism in the early 1920s, numerous studies and investigators have confirmed the association of hyperandrogenism and insulin resistance. In a landmark article Kahn et al.[1] reported on six patients with insulin resistance and acanthosis nigricans (which is an epiphenomenon of hyperinsulinemia) in whom insulin-binding studies demonstrated two abnormalities, namely, receptor abnormalities (type A) and anti-insulin-receptor antibodies (type B), the latter of which is associated with immunologic disease. Both syndromes have been associated with hyperandrogenism. In a subsequent study[2] a third variety of insulin resistance (type C), hyperandrogenism, and acanthosis nigricans were documented in obese women. Subjects in the type C syndrome lacked insulin receptor antibodies, and in these women (unlike the individuals with the type A syndrome) insulin binding to monocytes increased after short-term and long-term caloric restriction; thus the obesity of type C subjects was thought to be the mediator of insulin resistance.

Recognition of the remarkable association of hyperandrogenism with the hyperinsulinemia of these varied syndromes of insulin resistance led to the hypothesis that insulin may mediate ovarian androgen synthesis. Dunaif et al.[3] and Stuart et al.[4] demonstrated that insulin infusions to hyperandrogenic women produced a short-term increase in androstenedione. In a study that used glucose to stimulate further insulin production, Smith et al.[5] demonstrated a rise in androstenedione, testosterone, and dihydrotestosterone in hyperandrogenic women with insulin resistance. In addition, the magnitude of the rise in these androgens correlated strongly with the cumulative insulin response to the glucose challenge.

Both insulin and insulin-like growth factor-I receptors have been identified in human ovarian cells.[6] Barbieri et al.[7] studied the effect of insulin on incubated ovarian stromal fragments from patients with hyperandrogenism and found that insulin increased accumulation of testosterone and androstenedione. Thus in vivo and in vitro studies have confirmed that, in the presence of gonadotropins, insulin may induce ovarian androgen synthesis, either through the activation of its own receptors or via insulin-like growth factor-I receptors (the so-called receptor spillover phenomenon).[8]

In spite of such rationalization and the convincing nature of the data, there is an equally strong body of

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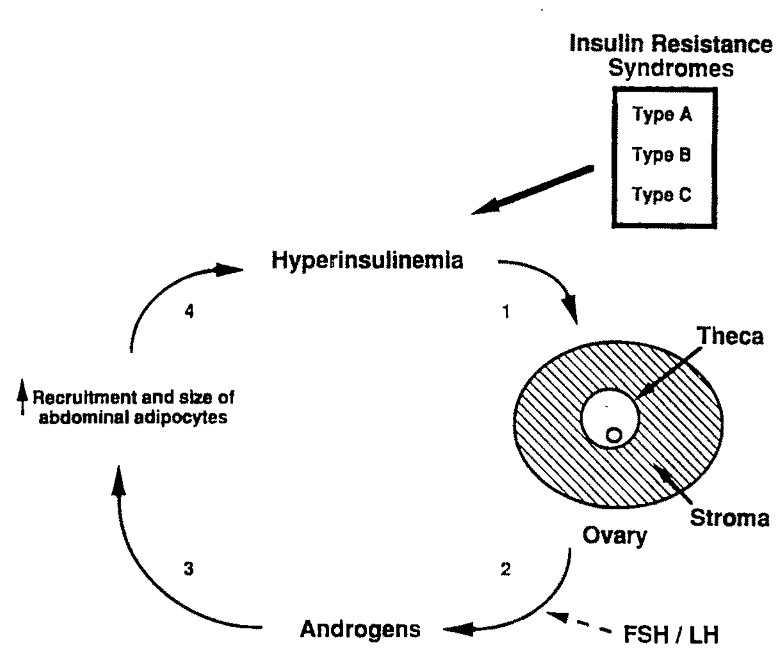
Insulin Resistance Syndromes
Type A
Type B
Type C
Hyperinsulinemia
1
Theca
Stroma
Ovary
2
FSH / LH
Androgens
3
↑ Recruitment and size of abdominal adipocytes
4

Fig. 1. Androgen-insulin connection: a unified concept. For explanation of *1* to *4*, see text. *LH*, Luteinizing hormone; *FSH*, follicle-stimulating hormone.

evidence that suggests a role for hyperandrogenism in the genesis of insulin resistance. For example, there are documented cases of luteomas and diabetes in which diabetes resolved after removal of the androgen-producing tumor.[9] Treatment of aplastic anemia with testosterone derivatives may result in glucose intolerance,[10] and in a recent case even danocrine (a modified androgen used to treat endometriosis) induced a temporary diabetic state.[11] Conversely, spironolactone (an antiandrogen) has been shown to lower insulin concentrations in women with polycystic ovary syndrome.[12]

Where is the connection?

It is not disputed that hyperandrogenism and hyperinsulinemia are linked, but where is the connection? Does hyperinsulinemia cause hyperandrogenism, or does hyperandrogenism induce a state of insulin resistance and subsequent hyperinsulinemia? Is there a missing link?

The missing link

In an excellent account of the biologic characteristics of regional fat distribution, Kissebah and Peiris[13] reviewed the information pertaining to regional body fat distribution. A summary of their data and the existing literature allows the following conclusions: (1) Obesity in women may be qualified according to its pattern of distribution: The more "android" the obesity (abdominal-visceral fat deposition), the greater the waist-to-hip ratio. "Gynoid" obesity is associated with femoral-gluteal fat deposition and a low waist-to-hip ratio. (2) Android obesity is, as its name implies, associated with "maleness." In a study of premenopausal women those with higher waist-to-hip ratio had a higher percent of free testosterone and lower sex hormone–binding concentrations.[14] In keeping with that observation, androgens have been shown to mediate the deposition of fat into abdominal-visceral depots (versus the femoral-gluteal region). For example, female-to-male transsexuals who are given testosterone accumulate fat in abdominal-visceral depots.[15] (3) Epidemiologically, Android obesity is linked to non-insulin-dependent diabetes mellitus.[16] (4) Morphologically and metabolically, fat cells in abdominal-visceral fat depots are distinct from femoral-gluteal fat cells. Whereas the femoral-gluteal depot is primarily a storage organ for female stresses such as pregnancy and lactation, the abdominal-visceral depot is a storage of energy reserves that can be easily and rapidly mobilized.[17] For example, abdominal-visceral fat cells exhibit higher rates of basal and catecholamine-induced lipolysis that results in greater free fatty acid concentrations.[18] (5) Androgens may mediate metabolic abnormalities via two additive mechanisms. First, androgens may directly impair hepatic and peripheral insulin action.[19] Second, by encouraging the deposition of abdominal-visceral fat with its resultant increase in free fatty acid flux, androgens impair hepatic insulin extraction and hepatic and peripheral insulin sensitivity.[20] These factors result in excess circulating insulin concentrations. Thus android obesity, which is a consequence of hyperandrogenism, leads to a state of insulin resistance and hyperinsulinemia that is independent of and additive to that of the degree of overweight.

The androgen-insulin connection: A unified concept

I propose that the following model provides a unified concept of the androgen-insulin connection (Fig. 1). Hyperinsulinemia, regardless of its cause and in the presence of permissive concentrations of gonadotropins, stimulates the ovarian thecal stromal compartments to produce androgens. These androgens not only directly influence insulin action but also increase the recruitment and size of abdominal adipocytes, which, through their specific metabolic qualities, lead to a further state of insulin resistance and hyperinsulinemia that is independent of the degree of overweight (i.e., via the high free fatty acid milieu suggested by Kissebah and Peiris[13]). This model can explain everything we know about polycystic ovary syndrome and its androgen-insulin connection. It explains how states of insulin resistance and hyperinsulinemia of diverse causes can be associated with hyperandrogenism (Fig. 1, 1). It explains how lowering gonadotropins (e.g., with the use of gonadotropin-releasing hormone analogs) may lower androgens (Fig. 1, 2) without necessarily altering insulin resistance (for example, it would not be expected to affect anti-receptor-antibody–mediated insulin resistance).[21, 22] It explains the commonly observed association between polycystic ovary syndrome and android obesity (Fig. 1, 3), and finally it explains how, under some circumstances, lowering androgen levels may indeed improve insulin sensitivity (Fig. 1, 4).

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Ultrasonographic screening for the Down syndrome fetus

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The usefulness of ultrasonography in detecting fetuses with Down syndrome in the second trimester has been the subject of considerable debate during the past 3 years. Conflicting reports have led to increasing confusion about the subject in the medical community and among the public. A review of the existing literature indicates that structural malformations that are chiefly cardiac are present in half of Down syndrome fetuses but are not consistently found during routine second-trimester ultrasonography. Although excess posterior nuchal skin-fold thickness is frequently present in infants with Down syndrome, it is also not consistently identified during second-trimester ultrasonographic examinations. Studies evaluating the efficacy of screening programs that are based on femur length shortening associated with Down syndrome have revealed markedly discrepant intercenter results. Although variations in methods may account for some of the observed variability, the magnitude of femoral shortening in Down syndrome suggests that this measurement may be only marginally useful. In summary, antenatal ultrasonography will allow for the detection of only those fetuses with Down syndrome who have multiple characteristic phenotypic features or anomalies. Moreover, current second-trimester ultrasonography does not appear to be either sensitive enough or specific enough to be used as a unique Down syndrome screening modality; however, it may be a useful adjunct to maternal age and serum biochemical markers in the assignment of risk for Down syndrome. (Am J Obstet Gynecol 1991;165:349-52.)

Key words: Ultrasonography, Down syndrome, screening

The incidence of Down syndrome is approximately 1 in 1000 live births.[1] This incidence is expected to increase modestly over the next 10 years as the number of women between 35 and 49 years of age increases.[2] However, the infants of women older than 34 will still account for only 8.6% of all live births and 39% of Down syndrome cases by the year 2000. Thus even a fully used Down syndrome screening program that is based on maternal age will fail to identify 61% of Down syndrome cases.[2]

The association of decreased maternal serum α-fetoprotein levels with fetal Down syndrome has now been well established. Risk determination that is based on a combination of maternal serum α-fetoprotein value and maternal age allows the detection of up to one-third of fetuses with Down syndrome and necessitates karyotype analysis in 5% of pregnant patients younger than 35.[3] However, Down syndrome risk assignment paradigms that use maternal age with a combination of maternal serum α-fetoprotein, human chorionic gonadotropin, and estriol values may identify 60% of fetuses with Down syndrome and necessitate karyotype analysis in only 5% of pregnant women.[4]

As a result of the explosive increase in the number of women who are undergoing diagnostic antenatal ultrasonographic studies, it was logical that ultrasonography also be evaluated in the quest for improved detection rates of Down syndrome. Unfortunately, initial reports have provided contradictory results and inconsistent methodologic approaches. An examination of the published experience of those who have used ultrasonographic indicators to detect fetuses with Down syndrome and speculations about the usefulness of this approach are the subjects of this review.

Why screen for fetal Down syndrome with ultrasonography?

Because most pregnant women now routinely undergo ultrasonographic evaluation in the second trimester, failure to use ultrasonographic information that could alter risk assignment of Down syndrome at the time of those examinations would be injudicious. However, this argument for the use of ultrasonographically derived data to determine the risk of Down syndrome requires that such data be reliably obtained, be effective in discriminating between fetuses with Down syndrome and unaffected fetuses, and be obtainable at a gestational age that is early enough to be useful clinically.

Ultrasonographic detection of Down syndrome fetus: Identification of coexistent anomalies

Certain malformations have been specifically associated with fetal Down syndrome. For example, 30% of

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fetuses with duodenal atresia have Down syndrome,[5] but only 3% to 8% of fetuses with Down syndrome have detectable upper gastrointestinal tract obstructions. Moreover, although the ultrasonographic diagnosis of duodenal atresia is readily made after 28 weeks' gestation, its consistent diagnosis may be difficult or impossible before 24 weeks.[5] Thus duodenal atresia, which is suggestive of Down syndrome, is not a useful ultrasonographic marker for most cases of Down syndrome.

The prevalence of aneuploidy in fetuses diagnosed with congenital heart defects has been reported to be 32%.[6] However, the vast majority of these fetuses have the far less prevalent trisomy 18 or trisomy 13.[6] This may reflect selection bias as a result of the more severe anatomic anomalies associated with fetuses with trisomy 18 or trisomy 13. The prevalence of congenital heart defects in fetuses with Down syndrome is generally accepted to be 40% to 50%; an atrioventricular canal defect is considered the "signature" cardiac lesion. However, although an atrioventricular canal defect is detectable on targeted four-chamber views of the heart, this lesion accounts for a minority of congenital heart defects that are associated with Down syndrome. Atrial and ventricular septal defects are more common and are not easily identified, even on targeted four-chamber views of the fetal heart. Thus, except in centers with advanced fetal echocardiography facilities, fetal congenital heart defects do not appear to be practical ultrasonographic markers for Down syndrome.

Renal anomalies are more common in aneuploid fetuses than in unaffected fetuses, but most fetuses with Down syndrome do not have major genitourinary tract anomalies. Benacerraf et al.[8] have recently described an association between mild fetal pyelectasis and fetal Down syndrome. They noted mild pyelectasis in 3.3% of unaffected fetuses compared with 25% of fetuses with Down syndrome. The usefulness of this observation is uncertain, because the prevalence of renal pelviceal dilatation in unaffected fetuses has been reported to be as high as 23%.[8]

Other malformations rarely associated with fetal Down syndrome include cystic hygromas, pleural effusions, and omphaloceles. Hygromas detected in utero are almost never accompanied by Down syndrome,[9] and the lesion is also extremely uncommon in fetuses with Down syndrome. Omphaloceles are associated with chromosomal abnormalities in 15% of cases, but only 12% of these are Down syndrome.[5] Furthermore, omphaloceles are rare in infants with Down syndrome. Pleural effusions, with or without hydrops, have been associated with Down syndrome,[10] but again infants with Down syndrome rarely have this abnormality.

In summary, screening for congenital malformations would result in a low detection rate for fetuses with Down syndrome because of the relative rarity of readily diagnosable coexistent malformations.

Ultrasonographic detection of Down syndrome fetus: Identification of typical phenotypic features

The phenotypic features of the infant with Down syndrome are sufficiently characteristic to have permitted the clinical diagnosis of Down syndrome in the prekaryotype era. Three of these features lend themselves to possible detection via ultrasonography: excess posterior nuchal skin-fold thickness, dysplasia of the midphalangeal ossification center of the fifth finger, and shortened length of long bones.

Excess nuchal skin-fold thickness. Benacerraf et al.[11] first described the association of ultrasonographically detected excess (≥ 6 mm) posterior nuchal skin-fold thickness with fetal Down syndrome. This sign was reported to be present in 9 of 21 fetuses with Down syndrome (43%) between 15 and 20 weeks' gestation but in $<0.1\%$ of unaffected pregnancies.[11] However, the reproducibility of this sign has been questioned.[12]

The efficacy of nuchal skin-fold thickness determinations in detecting the fetus with Down syndrome has also been assessed by several other investigators. Perrella et al.[13] identified excess nuchal skin-fold thickness in only 21% (3/14) of fetuses with Down syndrome, a false-positive rate of 9%. Hill et al.[14] observed excess nuchal skin-fold thickness in 9% (2/22) of fetuses with Down syndrome but did not report their false-positive rate. In contrast, Lynch et al.[15] identified excess nuchal skin-fold thickness in five of nine fetuses with Down syndrome (55%) but in none of their unaffected dizygotic twin controls.

The effect of gestational age on the extent of nuchal thickening has not been adequately evaluated. Therefore there is a need for autopsy confirmation of the prevalence of excess nuchal skin-fold thickness in second-trimester fetuses with Down syndrome. Toi et al.[12] evaluated 13 autopsy reports of abortuses with Down syndrome and reported an 18% prevalence of excess nuchal skin-fold thickness. However, the neck was not described in three cases and was described as being "normal" in six cases.

Clinodactyly and hypoplasia of midphalanx of fifth finger. Down syndrome is associated with a characteristic hypoplasia or dysplasia of the midphalanx of the fifth finger in 60% of infants; there is resultant curvature (clinodactyly) in 50%.[16] Benacerraf et al.[16] observed hypoplasia of the midphalangeal ossification center with concomitant clinodactyly of the fifth finger in three of four fetuses with Down syndrome between 17 and 20 weeks' gestation. In a subsequent study the same group compared the ratio of fifth digit/fourth digit midphalangeal ossification center lengths in 1024 normal fetuses and in 8 fetuses with Down syndrome. The mean ratios were 0.85 and 0.59, respectively. A ratio of ≤ 0.7 identified 6 of 8 fetuses with Down syn-

Table I. Efficacy of ultrasonographic screening for Down syndrome according to fetal femur length

Discriminator	*Down syndrome (No.)*	*Controls (No.)*	*Sensitivity (%)*	*False-positive rate (%)*	*Positive predictive value**	*Reference*
B/F >1.5 SD	35	349	51	7	1/97	18
M/E FL ≤0.91	28	192	68	2	1/21	19
B/F ≥1.8	15	45	40	2	1/40	Obstet Gynecol 1989;73:644-6
M/E FL ≤0.91	28	128	26	5	1/137	13
B/F >1.5 SD	22	286	36	7	1/129	14
M/E FL ≤0.91	22	286	50	15	1/213	14
B/F >1.5 SD	33	177	18	4	1/158	Obstet Gynecol 1989;74:726-9
M/E FL <0.91	33	177	15	10	1/473	Obstet Gynecol 1989;74:726-9
M/E FL ≤0.91	30	171	13	2	1/644	J Ultrasound Med 1989;8:657-60
M/E FL ≥0.90	6	428	50	6	1/85	Radiology 1990;174:837-9
B/F >1.28 SD	17	17	18	6	1/237	Obstet Gynecol 1990;75:186-8
B/F >1.5 SD	16	194	12	7	1/398	Am J Obstet Gynecol 1989;161:197-300
M/E <0.94	16	194	43	14	1/231	Am J Obstet Gynecol 1989;161:297-300
M/E ≤0.91	20	709	40	5	1/89	Radiology 1989;173:377-80
M/E <0.90	83	1340	24	6	1/186	Br J Obstet Gynaecol 1989;96:1373-8
M/E <0.91	49	572	14	6	1/305	Am J Obstet Gynecol 1990;162:1247-52

B/F, Ratio of biparietal diameter to femur length; *SD*, standard deviation; *M/E FL*, ratio of measured femur length to expected femur length for a given biparietal diameter.

*Based on prevalence of Down syndrome of 1 in 710 at 16 weeks.

drome; the false-positive rate was 18%. Postmortem confirmation of these prevalence data is not yet available. Moreover, given the technical expertise and state-of-the-art equipment required to make measurements (1 to 4 mm) that approach or exceed the lateral resolution of many ultrasonographic machines, this approach is unlikely to have immediate widespread application.

Biometric differences in long-bone lengths between Down syndrome fetuses and unaffected fetuses. Lockwood et al.[18] suggested the use of routine biometry measurements in the ultrasonographic identification of fetuses at risk for Down syndrome. They retrospectively analyzed ultrasonographically derived biometry differences in fetuses with Down syndrome and in unaffected control fetuses between 15 and 23 weeks' gestation. No statistically significant differences were noted in the cephalic index or biparietal diameter between the groups at any gestational age. However, a significant degree of femur length shortening in affected fetuses was noted. Moreover, the best discriminator of the fetus with Down syndrome was the ratio of biparietal diameter to femur length. When a cutoff value of 1.5 SD above the unaffected population biparietal diameter/femur length mean is considered, at least 50% of fetuses with Down syndrome could be identified in 6% of the total population.

Benacerraf et al.[19] evaluated femur length as a function of biparietal diameter in fetuses with Down syndrome versus control fetuses. They used a ratio of the measured femur length to expected femur length for a given biparietal diameter. With a measured femur length/expected femur length cutoff of 0.91, they identified 68% of fetuses with Down syndrome (false-positive rate 2%). Subsequent published reports have used either the biparietal diameter/femur length at a given gestational age or the measured femur length/expected femur length for a given biparietal diameter. Their findings have been highly variable (Table I).

The explanation for such different results is unclear. Small numbers of Down syndrome and control fetuses, as well as differences among manufacturers of ultrasonographic equipment, models, and transducers, are present. Operator experience and measurement technique may also vary significantly among centers. Furthermore, longer ascertainment periods for Down syndrome cases compared with control populations appear to be the rule. This latter bias could result in less operator experience, inferior ultrasonographic equipment, and reduced ultrasonographic resolution for the earlier Down syndrome cases compared with controls at some of the centers.

Biologic differences in long-bone lengths between fetuses with Down syndrome and unaffected fetuses may not be of sufficient magnitude to permit discrimination by ultrasonography in the second trimester, as a result of the limits of lateral resolution in the equipment available at this time. FitzSimmons et al.[20] have assessed postmortem fetal long-bone lengths, either directly or with radiographic studies, in fetuses with Down syndrome and in unaffected fetuses. They found small but significant differences between the femur lengths of Down syndrome fetuses and those of unaffected fetuses. However, a ratio of measured femur length to expected femur length of <0.91 permitted the detection of only 16% of Down syndrome abortuses in their study. The authors did not indicate this ratio's false-positive rate or whether alternate cutoff ratios improved sensitivity.

Interestingly, FitzSimmons et al. found that upper-extremity long-bone lengths were more severely affected than lower-extremity lengths in fetuses with Down syndrome. They found that approximately 30% of abortuses with Down syndrome had humeral lengths <2 SD below the mean of those that were unaffected. This suggests that humeral length may be a better discriminator of fetal Down syndrome than femur length.

Comment

The utility of ultrasonographic Down syndrome detection schemes should be judged by whether the pertinent ultrasonographic markers are reliably obtained and by whether they efficiently discriminate between fetuses with Down syndrome and euploid fetuses at a gestational age early enough to be clinically useful. Unfortunately, none of the currently proposed approaches satisfactorily fulfills all these requirements. Screening for characteristic congenital malformations would result in a low detection rate of Down syndrome, and many of these cases would be identified only after 24 weeks' gestation. Second-trimester ultrasonographic identification of typical Down syndrome phenotypic features is hindered by the subtlety of the findings, which mandate significant technical expertise. Furthermore, there is considerable overlap of these features with the unaffected population, and the effects of gestational age exacerbate this overlap.

Second trimester ultrasonography does not as yet appear to have a useful primary role in Down syndrome screening. We believe, however, that fetal biometry and assessment of nuchal skin-fold thickness may (particularly if measurements of the upper extremity are included) be an adjunct to maternal age, maternal serum α-fetoprotein, and human chorionic gonadotropin values in the assignment of risk for Down syndrome.

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Clinical Articles

The fetal-pelvic index: A method of identifying fetal-pelvic disproportion in women attempting vaginal birth after previous cesarean delivery

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In 1986 the fetal-pelvic index was introduced as an accurate method of prospectively identifying the presence or absence of fetal-pelvic disproportion. The concept of the fetal-pelvic index is one in which the fetal head and abdominal circumferences (ultrasonographic mensuration) are compared with the respective maternal pelvic inlet and midpelvic circumferences (x-ray pelvimetry). The purpose of this study is to evaluate the efficacy of this index as a predictor of fetal-pelvic disproportion in gravid women attempting vaginal birth after previous cesarean delivery. Findings of the fetal-pelvic index are compared with those of two other means of identifying fetal-pelvic disproportion (Colcher-Sussman x-ray pelvimetry and ultrasonographically derived estimated fetal weight of ≥4000 gm). Of the 65 prospective study participants, 18 (28%) failed to progress in labor, and a repeat cesarean delivery was required. Forty-seven of the 52 patients with a negative fetal-pelvic index value were delivered vaginally (negative predictability = 0.90). Of the five with false-negative fetal-pelvic index values, four fetuses persisted in an occipitoposterior position and failed to progress in labor. All 13 patients with a positive fetal-pelvic index value failed to progress in labor and required a cesarean delivery (positive predictability = 1.00). In contrast, when used alone, neither x-ray pelvimetry nor ultrasonography-determined estimated fetal weight of ≥4000 gm provided accurate identification of fetal-pelvic disproportion. (AM J OBSTET GYNECOL 1991;165:353-8.)

Key words: Fetal-pelvic disproportion, fetal-pelvic index, vaginal birth after previous cesarean delivery

Since 1970 the dramatic increase in the rate of cesarean section has made it the most frequently performed major operation in the United States.[1-3] Furthermore, *elective repeat cesarean* has become the most common indication for cesarean delivery.[4, 5] Although the safety of vaginal birth after previous cesarean delivery has been well documented,[6-10] elective repeat cesarean remains the rule in most labor and delivery units.

Flamm et al.[11] reported on the frequency of vaginal birth after previous cesarean delivery and found it to be related to the indication for the prior cesarean delivery. In their multicenter study of 1776 labor trials, women with failure to progress or cephalopelvic disproportion as the documented indication for cesarean delivery had the lowest success rate (65%) for subsequent vaginal birth whereas those women with breech presentation as the indication had the highest success rate (88%). Although approximately 75% of women attempting vaginal birth after cesarean delivery reportedly are successful,[11] a method of prospectively identifying fetal-pelvic disproportion could prevent prolonged unsuccessful labor trials in the majority of women destined to fail the attempt.

In 1986 the fetal-pelvic index was introduced as an accurate method of prospectively identifying the presence or absence of fetal-pelvic disproportion.[12] The concept of the fetal-pelvic index is one in which the fetal head and abdominal circumferences (passenger) are compared with the respective maternal pelvic inlet and midpelvic circumferences (passage). The purpose of this study is to evaluate the accuracy of the fetal-pelvic index as a predictor of fetal-pelvic disproportion and delivery outcome in gravid women attempting vaginal birth after previous cesarean delivery. Results of the fetal-pelvic index data are compared with those of two other means of identifying fetal-pelvic disproportion (Colcher-Sussman x-ray pelvimetry[13] and ultra-

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Table I. Results of measurement data*

			Ultrasonography (cm)		*X-ray pelvimetry (cm)*	
Delivery outcome	*No.*	*Birth weight (gm)*	*Fetal head circumference*	*Fetal abdomen circumference*	*Maternal pelvic inlet circumference*	*Maternal midpelvis circumference*
Cesarean delivery	18	3486 ± 393	33.0 ± 1.6	35.1 ± 2.1	37.1 ± 2.1	33.8 ± 2.4
Vaginal delivery	47	3325 ± 514	32.8 ± 1.2	33.7 ± 1.9	39.6 ± 2.4	36.5 ± 2.0
p value†		NS	NS	<0.025	<0.001	<0.001

*Mean ± SD.

†*t* Test.

Table II. Results of study data in women attempting vaginal delivery after previous cesarean delivery

	Ultrasonography		*X-ray pelvimetry*		*Fetal pelvic index*	
Delivery outcome	*Estimated fetal weight ≥ 4000 gm*	*Estimated fetal weight < 4000 gm*	*Contracted maternal pelvis*	*Adequate maternal pelvis*	*Positive*	*Negative*
Cesarean delivery	2	16	3	15	13	5
Vaginal delivery	2	45	0	47	0	47
Sensitivity	0.11		0.17		0.72	
Specificity	0.96		1.00		1.00	
p value*	NS		<0.02		<0.00001	

*Fisher's exact test.

sonographically determined estimated fetal weight of ≥4000 gm).[14]

Material and methods

Seventy-four gravid women between 35 and 43 weeks' gestation with a desire for vaginal birth after previous cesarean delivery, were entered into the study between Nov. 1, 1988, and Jan. 31, 1990. Nine women were excluded. Of these nine, five did not have x-ray pelvimetry performed, two did not have fetal ultrasonographic measurements performed, and two had an inadequate labor trial before cesarean delivery (cervical dilatation <5 cm). Of the 65 study participants, 64 fetuses presented in cephalic position, and one was a frank breech presentation. Spontaneous labor was observed in 58 women, and seven received intravenous oxytocin-stimulated labor (five inductions, two augmentations). All patients were monitored with a fetal scalp electrode and an intrauterine pressure catheter.

An adequate labor trial was defined as (1) uterine contractions of at least 40 mm Hg in intensity and 60 seconds in duration, (2) a frequency of at least three contractions in 10 minutes, and (3) cervical dilatation of ≥5 cm for a minimum of 2 hours.

Fetal ultrasonographic evaluations were performed in the labor rooms, by means of a portable ADR 4000 S/L linear-array scanner with a 3.5 MHz transducer calibrated to a sound velocity in soft tissue of 1540 m/sec (Advanced Technology Labs Inc., Tempe, Ariz.). Diameters of the fetal head—outer-to-inner biparietal, outer-to-outer transverse, and outer-to-outer anterior-posterior—were determined by built-in electronic calipers applied to a freeze-frame image of the plane that included the thalamus, the septum cavum pellucidum, and a symmetrically aligned midline echo. Fetal abdominal diameters were also determined from a freeze-frame image that visualized the plane perpendicular to the fetal spine at the level of the portal vein bifurcation and stomach. By means of electronic calipers the anterior-posterior diameter was measured from the outer aspect of the fetal spine to the outer anterior abdominal wall. The outer-to-outer transverse diameter was measured 90 degrees to the anterior-posterior diameter.

With regard to the maternal pelvic measurements, respective anteroposterior and transverse diameters of the inlet and midpelvis were measured by x-ray pelvimetry (Colcher-Sussman technique).[13] Maternal pelvimetry was performed in each case after 37 weeks' gestation and before either the active phase of labor or intravenous oxytocin administration. This method consists of simple positioning of the patient in two views (lateral and anteroposterior). With each view an appropriately positioned ruler is included on the respective film for rapid and direct mensuration of the above-mentioned diameters. On the basis of a sum of the transverse and anterior-posterior diameters of the pelvic inlet as well as that of the midpelvis, an adequate pelvic size is defined as being >22 cm for the inlet and >20 cm for the midpelvis.[13]

The method of determining fetal-pelvic index values is as follows:[12] Individually measured anteroposterior

Table III. Results of delivery outcome and fetal-pelvic index data relative to indication for previous cesarean section

		Indication for previous cesarean							
		Cephalopelvic disproportion/failure to progress		*Fetal distress*		*Breech*		*Other*	
Delivery outcome	*No.*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*
Cesarean delivery	18	9(2*)	4(3)	4(1)	1(1)	0	0	0	0
Vaginal delivery	47	0	18	0	9	0	14[1†]	0	6(2)
Sensitivity		0.69		0.80		NA		NA	
Specificity		1.00		1.00		1.00		1.00	
Predictability		0.87		0.93		1.00		1.00	
p Value‡		<0.00001		0.005		NA		NA	

NA, Not available.

*Number in parentheses are patients with persistent occipitoposterior fetal presentation.

†Persistent frank breech fetal presentation.

‡Fisher's exact test.

and transverse diameters of the fetal cranium, fetal abdomen, maternal pelvic inlet, and maternal midpelvis are used to compute respective circumferences of the fetal head (HC), fetal abdomen (AC), maternal pelvic inlet (IC), and maternal midpelvis (MC) by the equation: $C = (TD + APD) \times 1/2\ \pi$. On the basis of four circumference differences between the fetus and the maternal pelvis (HC-IC, HC-MC, AC-IC, AC-MC), a fetal-pelvic index value is derived from the sum of the two most positive circumference differences. A positive fetal-pelvic index value identifies a fetus that is larger than the maternal pelvis (presence of fetal-pelvic disproportion), and a negative fetal-pelvic index value identifies a fetus that is smaller than the maternal pelvis (absence of fetal-pelvic disproportion).

To provide comparative data, two other means of identifying fetal-pelvic disproportion were analyzed (Colcher-Sussman x-ray pelvimetry[13] and ultrasonographically determined estimated fetal weight of ≥4000 gm according to Shepard tables[14]). Results of the measurement and fetal-pelvic index data were not available to the clinicians managing the labors, and the investigators were not informed of the delivery outcomes. All three methods were compared with regard to delivery outcome and their respective accuracy of predicting the presence or absence of fetal-pelvic disproportion. This study was reviewed and approved by the institutional review board. Data were analyzed by Fisher's exact, χ^2, and independent two-tailed t tests, where appropriate.

Results

Of the 65 study participants, 18 (28%) failed to progress in labor and required a repeat cesarean delivery. Forty-seven (72%) were delivered vaginally without operative intervention. The two groups were comparable with regard to maternal age and parity. However, when compared with the vaginal delivery group, a greater proportion of nonwhite patients was observed in the cesarean group. The mean gestational age at the time of delivery was 39.5 ± 1.8 weeks for the cesarean group and 39.1 ± 1.4 weeks for those delivered vaginally.

Results of the mean birth weight, fetal ultrasonographic, and maternal x-ray pelvimetry measurement data are given in Table I. Mean birth weights and fetal head circumferences for both groups were similar. However, when compared with those who were delivered vaginally, the mean fetal abdominal circumference was significantly larger ($p < 0.025$) and the mean maternal inlet and midpelvic circumferences were significantly smaller ($p < 0.001$) in the cesarean group. Moreover, the measurement parameters contributing most often to observed fetal-pelvic disproportion were the respective fetal abdominal and maternal midpelvic circumferences, not the fetal head circumferences.

Delivery outcomes for the three methods tested are given in Table II. When ultrasonographically determined estimated fetal weight was used alone, with ≥4000 gm as the threshold weight for fetal-pelvic disproportion, the sensitivity was 0.11, specificity 0.96, and overall predictability 0.72. When x-ray pelvimetry was used alone, with a contracted maternal pelvis defined as either <22 cm for the inlet or <20 cm for the midpelvis, the sensitivity was 0.17, specificity 1.00, and overall predictability 0.77. Because of their low sensitivities, neither of these techniques provided accurate identification of fetal-pelvic disproportion.

Of the 18 women requiring repeat cesarean delivery

Table IV. Summary of fetal-pelvic index studies

			Delivery type					
			Operative					
			Cesarean		*Vaginal*		*Spontaneous vaginal*	
Study	*Year*	*No.*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*	*Positive fetal-pelvic index*	*Negative fetal-pelvic index*
Original[12]	1986	75	23	4(4†)	4	0	0	44
Fetal macrosomia[33]	1988	34	12	1(1)	5	0	1	15
Labor induction[34]	1988	49	10	2(2)	2	0	0	35
Labor augmentation[35]	1988	46	14	5(4)	3	2(2)	1	21
Vaginal birth after previous cesarean delivery	1991	65	13	5(4)	0	0	0	47

*Reason for operative delivery: cesarean, failure to progress in active phase (cervical dilatation >5 cm); operative vaginal, difficult midforceps or shoulder dystocia.

†Numbers in parentheses are patients with persistent occipitoposterior fetal presentation.

for failure to progress in labor, 13 demonstrated positive fetal-pelvic index values (sensitivity, 0.72) and five had negative index values (false negative). The five cases with false-negative fetal-pelvic index values deserve further discussion. Four of these five fetuses persisted in an occipitoposterior position and failed to progress in labor. The fifth fetus presented in an occipitoanterior position and had a birth weight of 3920 gm. Although no cephalopelvic disproportion was observed, a small degree of abdominal-midpelvic disproportion was present, and cervical dilation stopped at 6 cm.

All 47 vaginally delivered patients had negative fetal-pelvic index values (specificity, 1.00; negative predictability, 0.90). Moreover, all 13 women with positive fetal-pelvic index values required a repeat cesarean delivery (positive predictability, 1.00; Table II).

Delivery outcomes and fetal-pelvic index data relative to the indication for the previous cesarean section are given in Table III. The indications were divided into the following categories: cephalopelvic disproportion or failure to progress, fetal distress, breech presentation, and other (placenta previa, $n = 1$; abruptio placentae, $n = 2$; preeclampsia-eclampsia, $n = 2$; and unknown, $n = 1$). Of the 31 women with a previous cesarean delivery because of cephalopelvic disproportion or failure to progress, 18 (58%) were delivered vaginally. In contrast, when the previous cesarean section was performed because of fetal distress, nine (64%) of 14 were delivered vaginally. Of the 20 women with a history of previous cesarean section as a result of breech presentation and "other" indications, all were delivered vaginally. Sensitivity, specificity, and predictability of the fetal-pelvic index, relative to the indication for the previous cesarean section, are given in Table III.

Overall, malposition was observed in 10 fetuses—nine occipitoposterior presentation and one frank breech (Table III). Of the nine fetuses presenting in persistent occipitoposterior position, seven failed to progress in labor and a cesarean section was necessary (fetal-pelvic index values ranged from +1.1 to −5.0); two were delivered vaginally (fetal-pelvic index values were −6.9 and −8.8). The fetus presenting in a frank breech position was delivered vaginally with a fetal-pelvic index value of −7.8.

Comment

In management of a pregnant woman with a history of a previous cesarean delivery, the following three questions should be addressed: (1) What is the appropriate mode of delivery for this pregnancy? (2) When vaginal delivery is being considered, will labor compromise the safety of the fetus and mother? (3) Can the presence or absence of fetal-pelvic disproportion be identified prospectively in women attempting vaginal birth after previous cesarean delivery?

The answer to the first question dates back to Cragin's 1916 article, entitled "Conservatism in Obstetrics," in which he stated "The usual rule is once a cesarean always a cesarean."[15] Although Cragin's usual rule was not supported with data, it was applicable to the times in which he practiced. Subsequently, fear of rupture of a previous uterine scar was universally accepted.[16] By 1978, approximately 99% of American women with a history of a primary cesarean section underwent an automatic repeat cesarean of subsequent deliveries.[4] Thus for more than 60 years obstetricians believed automatic repeat cesarean to be the appropriate mode of delivery for women with a history of primary cesarean section. In 1991, this belief may not be appropriate for the majority of women, and vaginal delivery should be considered in selected cases.

Sensitivity	Specificity	Predictability	
		Positive	Negative
0.87	1.00	1.00	0.92
0.94	0.94	0.94	0.94
0.86	1.00	1.00	0.95
0.71	0.95	0.94	0.75
0.72	1.00	1.00	0.90

The answer to the second question has been addressed extensively during the past two decades. Since 1970 the cesarean section rate has increased from 5.5% to 24.4%, making cesarean section the most frequently performed major surgical procedure in United States hospitals.[17] Concern over the dramatic rise in the cesarean section rate in the United States and the relative increase in the number of elective repeat cesareans led to a formal reexamination of the beliefs and fears governing cesarean delivery.[18] Four diagnostic categories were identified as primarily responsible for the increasing cesarean rate: repeat cesarean (35%), dystocia (30%), breech presentation (10% to 15%), and fetal distress (10% to 15%). Although the threat of malpractice was thought to be a consideration, the National Institutes of Health Consensus Report panel could not accurately assess the impact of defensive medical practices.

Because of the lack of objective justification for the overall increasing cesarean section rate, attempts have been made to reduce it. Moreover, a particular focus has been on vaginal birth after previous cesarean delivery. During the past 5 years, a number of investigators have addressed the prevailing negative attitude toward vaginal birth after previous cesarean delivery with reports on uterine rupture,[19] labor trials and vaginal birth after previous cesarean delivery,[11, 20] new American College of Obstetricians and Gynecologists guidelines for vaginal birth after previous cesarean delivery,[21] programs to lower cesarean section rates,[22] perinatal mortality rate after vaginal birth after previous cesarean delivery,[23] vaginal delivery after more than one previous cesarean,[24, 25] maternal risks in vaginal birth after previous cesarean delivery,[26] vaginal birth after previous cesarean delivery in cases of macrosomic fetuses,[27] and vaginal birth after previous cesarean delivery in cases of multifetal gestations and breech fetuses.[28] On the basis of these reports, the safety of a labor trial and subsequent successful vaginal delivery of the majority of women attempting vaginal birth after previous cesarean delivery has been established. As a result, automatic repeat cesarean section as the primary cause of the rising cesarean birth rate is being challenged.[29-32]

The answer to the third question is the principal objective of this report. When a vaginal birth after previous cesarean section is considered, the most important issue is appropriate patient selection. Central to this selection process is the ability to identify fetal-pelvic disproportion. This issue has not been addressed adequately because an accurate method of identifying the presence or absence of fetal-pelvic disproportion has not been available.

In 1986 we introduced the fetal-pelvic index as an accurate method of prospectively identifying the presence or absence of fetal-pelvic disproportion.[12] In three subsequent studies we reported on the reproducible accuracy of this method in women with the following conditions considered at high risk for cesarean section: (1) macrosomic fetus,[33] (2) labor induction,[34] and (3) an abnormal labor pattern requiring augmentation.[35] In this study we report on the efficacy of fetal-pelvic index as a method of prospectively identifying the presence or absence of fetal-pelvic disproportion in women attempting vaginal birth after previous cesarean delivery. On the basis of these data, we believe the fetal-pelvic index provides an accurate method of identifying the presence or absence of fetal-pelvic disproportion in these women. With this approach, proper selection of those women who will successfully be delivered vaginally can be made. When the managing obstetrician is able to identify the absence of fetal-pelvic disproportion, a more active management of labor can be pursued with confidence. Conversely, when the fetal-pelvic index is positive, prolonged unsuccessful labor trials can be prevented in those women destined to fail. The impact (financial, emotional, and medical) of being able to identify fetal-pelvic disproportion and predict delivery outcome in more than 90% of patients with a previous cesarean delivery is potentially enormous.

Findings of this study support those of our previous reports in which various x-ray pelvimetry measurement criteria and various ultrasonographically derived estimated fetal weights, when used alone, do not predict fetal-pelvic disproportion accurately.[12, 33-35] In Table IV, a summary of data from the four previous fetal-pelvic index studies and the present study is given. Overall, the sensitivity of the fetal-pelvic index is 0.82, specificity is 0.99, positive predictability is 0.98, and negative predictability is 0.90. Of the 19 cases with a negative fetal-pelvic index requiring a cesarean section (false negatives), 17 fetuses demonstrated a persistent occipitoposterior position and failed to progress in labor.

Although the predictive accuracy of the fetal-pelvic index in each of our studies has been highly reproducible, our findings need to be substantiated by other investigators before a formal recommendation can be made to all clinicians. Therefore we hope more centers will evaluate the efficacy of the fetal-pelvic index in women with all conditions at high risk for cesarean delivery.

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Acute myocardial infarction associated with prostaglandin E_2

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Prostaglandin E_2 is rarely associated with serious maternal side effects when used for second-trimester pregnancy termination. Acute myocardial infarction complicating therapeutic pregnancy termination with prostaglandin E_2 in a patient with chronic glomerulosclerosis and severe hypertension is reported. (AM J OBSTET GYNECOL 1991;165:359-60.)

Key words: Prostaglandin E_2, myocardial infarction, pregnancy termination

Prostaglandin E_2 (PGE_2) is the preferred agent for second-trimester pregnancy termination, replacing instrumented evacuation of the uterus and intraamniotic injection of abortifacient agents. It is also useful for cervical ripening and induction of labor in pregnancies complicated by serious maternal illness. Side effects are usually mild and include nausea, vomiting, pyrexia, diarrhea, and occasionally hypotension. We report a case of severe hypotension and acute myocardial infarction associated with PGE_2 in a patient undergoing therapeutic termination of pregnancy.

Case report

A 38-year-old black woman, gravida 3, para 0-0-2-0, with chronic hypertension and renal insufficiency as a result of chronic glomerulosclerosis was seen by her physician with a 2-day history of symptoms consistent with congestive heart failure. Obstetric history was remarkable for two fetal deaths at 27 weeks' gestation because of preeclampsia. Initial evaluation revealed a blood pressure of 260/140 mm Hg, bilateral rales, pretibial edema, and hyperreflexia. Electrocardiogram demonstrated normal sinus rhythm with a rate of 85 beats/min. Mild cardiomegaly and slight pulmonary edema were present on chest x-ray film. Echocardiogram revealed moderate concentric left ventricular hypertrophy with mild global hypokinesis. A gestation of 19 weeks was estimated by ultrasonography. Significant laboratory values included blood urea nitrogen, 51 gm/24 hours; creatinine, 5.0 mg/dl; hemoglobin, 6.7 gm/dl; and uric acid, 6.1 mg/dl. Liver function test results and platelet count were normal. Magnesium sulfate was given, a nitroprusside infusion was started, and the patient was transported to our hospital with the diagnosis of possible superimposed preeclampsia.

The nitroprusside was subsequently gradually withdrawn in our intensive care unit, and the patient was stabilized on a regimen of a hydralazine infusion with intermittent intravenous methyldopa and furosemide. Hemoglobin was increased to 9.4 gm/dl after a transfusion of 2 units of packed red blood cells. Liver function test results, platelet count, and urine output remained normal, with a urine protein of 2.3 gm/24 hours. Two days after admission, a therapeutic termination of pregnancy was requested by the patient after she was counseled regarding the maternal risk of continuing the pregnancy.

A 20 mg PGE_2 vaginal suppository was administered while the patient was continuously monitored in the cardiac care unit. Blood pressure was 180/98 mm Hg while she was receiving a hydralazine infusion of 8 mg/hr with no other antihypertensives (except intravenous furosemide) given within the previous 22 hours. Approximately 30 minutes after administration, the patient became hypotensive with bradycardia, and she experienced cardiovascular collapse. Rhythm strips documented transient complete heart block followed by ventricular fibrillation. The heart was defibrillated, and she was then stabilized with vasopressors, lidocaine, an intraaortic balloon pump, and ventilatory support. A 12-lead electrocardiogram revealed an acute inferior wall myocardial infarction, which correlated with a new inferior wall hypokinesis on repeat echocardiogram. Cardiac catheterization revealed angiographically normal coronary arteries. Hemodynamic status improved rapidly, and the patient was off assisted ventilation, balloon pump, and vasopressors within 24 hours. Spontaneous delivery of the fetus occurred 8 hours after the myocardial infarction. Urine output was maintained with only slight elevation of the creatinine level to 7 mg/dl, and recrudescent hypertension was controlled with oral medications. The remainder of the hospital course was uncomplicated.

Comment

Severe cardiovascular complications associated with PGE_2 are rare. PGE_2 is rapidly absorbed after vaginal administration and can produce hypotension as a result of direct arteriolar vasodilation. Sampson et al.[1] re-

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ported a mild decrease in mean arterial pressure after PGE_2 administration for midtrimester pregnancy termination in patients with severe preeclampsia receiving antihypertensive agents. None of these patients had serious complications.

The time interval between PGE_2 administration and the circulatory collapse and myocardial infarction in our patient would imply a relationship between these events. Preexisting coronary artery disease did not complicate this case. We propose that PGE_2 acted synergistically with the antihypertensive therapy to cause profound hypotension, resulting in coronary hypoperfusion and myocardial infarction. PGE_2 has a short biologic half-life, and the rapid recovery from cardiovascular shock observed in this case also is compatible with a causal relationship. PGE_2-induced coronary artery spasm seems unlikely because it has been shown to possess coronary artery relaxant effects.[2] In any case PGE_2 should be used with caution in patients receiving vasoactive antihypertensive medications, especially when preexisting cardiac disease or risk factors are present.

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CA 125 regression: A model for epithelial ovarian cancer response

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The rate of decline of CA 125 in effectively treated epithelial ovarian cancer is described by the exponential regression curve CA 125 = EXP [$i - s$ (days after surgery)]. In this equation i, the y-axis intercept, measures initial tumor burden whereas s, the slope of the regression curve, is determined by the extent of cytoreductive surgery and the subsequent response to chemotherapy. Departure from the regression curve uniformly results in progressive disease. In patients whose cancers had been completely removed, we calculated the mean half-life of CA 125 to be 10.4 days (range 4 to 21). In this case $s = 0.0835$ and characterizes the ideal regression rate. The model predicts that high-dose cisplatin chemotherapy ($s = 0.0671$) is more effective than low-dose cisplatin ($s = 0.0380$) ($p < 0.03$) in eliminating residual cancer. Because s can be calculated within 2 to 3 months of treatment and then compared with s for the ideal regression curve and with the values of s reported for standard chemotherapy, evaluation of any new treatment protocol can be facilitated with this method. (AM J OBSTET GYNECOL 1991;165:360-7.)

Key words: CA 125, exponential regression, chemotherapy

Ovarian carcinoma is the fourth most common cause of death among American women.[1] Aggressive surgical cytoreduction and subsequent cisplatin-based chemotherapy have helped prolong disease-free survival for women with advanced disease.[1,2] Our ability to monitor disease status during therapy has been facilitated by measurement of the tumor-associated antigen CA 125. Bast et al.[3] and others[4,5] have shown that changes in serum CA 125 levels reflect progression or regression of epithelial ovarian cancer >90% of the time. That is to say, increases in serum CA 125 levels reflect disease progression whereas declines in serum CA 125 levels reflect a response to therapy. Thus by monitoring CA

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125 levels during treatment, it has been possible to predict response to therapy in the absence of clinically measurable disease and to prognosticate survival.[6-10] Most clinical trials still rely on second-assessment celiotomy to determine the initial effectiveness of chemotherapy because survival data, which are the only realistic end point of treatment efficacy, can take years to generate. Simply measuring serum CA 125 levels before second-assessment celiotomy is not helpful because the negative predictive value in a patient with stage III or stage IV epithelial ovarian cancer is only 50%.[11] Reliance on second-assessment celiotomy is costly. Awaiting accrual of survival data can be inefficient. Such an approach may result in missed opportunities to initiate phase II drug studies in women with a small tumor burden before depletion of marrow reserves. This study was designed to develop a model of tumor response by considering the serum CA 125 level as a continuous variable that is reflective of tumor growth or regression for those epithelial ovarian cancers that are marker positive. As the model was developed, we used it to characterize the initial clinical responsiveness of CA 125–secreting epithelial ovarian cancers. The model can be used to compare the relative efficacy of different therapeutic regimens. In a separate communication we will show how initial responsiveness is predictive of findings at second-look celiotomy and translates into overall survival.

Material and methods

Sixty-four patients with advanced ovarian epithelial carcinoma who were treated at the University of California, Irvine (UCI), or its affiliated hospitals, beginning in January 1986 through June 1990, form the population of this study. Women treated during this time interval who were marker negative or who did not have sequential measurements of serum CA 125 levels beginning early in therapy could not be evaluated. Patient characteristics and follow-up are summarized in Table I. The average age at diagnosis was 62.6 years (range 43 to 80). Eighty-four percent of patients in this study had stage III or stage IV disease. Ninety-four percent of the lesions were grade 2 or 3. We also included two patients with dual primary endometrial and ovarian lesions that were confined to the pelvis and were CA 125 positive. All histologic types of epithelial ovarian cancer were included. Seventeen patients died of progressive or recurrent ovarian carcinoma during the study interval. Three patients died apparently of causes other than tumor recurrence 6.7, 6.8, and 35.9 months into therapy without evidence of disease. The mean follow-up for all patients was 15.6 months (range 2 to 48).

Table I. Background data (64 patients)

Age at diagnosis (yr, mean and range)	62.6 (43-80)
Followup (days, mean and range)	468 (60-1445)
Deaths	
From cancer	17
Other, without cancer	3
FIGO stage	
IIA, IIB, IIC	6
IIIA, IIIB, IIIC	42
IV	12
Unknown	2
Dual primary	2
Grade	
1	4
2	21
3	39
Histologic type	
Serous	41
Clear cell	2
Endometrioid	4
Undifferentiated	17

Treatment consisted of biopsy only for seven patients, all operated on at outside facilities and referred to UCI for chemotherapy. In 13 patients cancer was completely resected whereas 26 underwent cytoreduction to residual cancer >2 cm and 18 had residual disease <2 cm. All patients were treated with cisplatin-based chemotherapy. The chemotherapy regimens planned were as follows: CAP [cisplatin, approximately 50 mg/m^2; doxorubicin (Adriamycin, Adria, Columbus, Ohio), approximately 50 mg/m^2; and cyclophosphamide (Cytoxan, Bristol-Myers, Syracuse, N.Y.), approximately 50 mg/m^2] in eight patients for eight cycles; low-dose cisplatin, approximately 50 mg/m^2, and Cytoxan, approximately 500 mg/m^2) in 30 patients for eight cycles; high-dose cisplatin approximately 100 mg/m^2, and Cytoxan, approximately 1000 mg/m^2, in 21 patients for four cycles. Other treatments included cisplatin, approximately 75 mg/m^2, and primary intraperitoneal cisplatin or carboplatin plus intravenous Cytoxan or Adriamycin, or both, for five patients. Twenty-three of the patients included in the study were randomized to treatment regimens on a Gynecologic Oncology Group protocol designed to compare high-dose and low-dose cisplatin chemotherapy. Four patients, two on the low-dose cisplatin regimen and two on CAP, did not complete the planned chemotherapy because of progression of disease.

Statistical evaluations included least-squares regression analysis of CA 125 data as a function of time. Comparison of means was achieved by the unpaired Student t test requiring $p < 0.05$ for statistical significance. When categorical variables were analyzed, we used χ^2 with Yates' correction as indicated.

Model development

Levels of CA 125 were plotted against time with the day of surgical cytoreduction recorded as time 0. If a

Table II. Model development

Condition	r	p	i	s
Linear				
Preoperative*–first normal†	−0.86	0.030	6460	128
Preoperative–no normal‡	−0.91	0.031	7243	190
Pretreatment–first normal	−0.87	0.058	4692	90
Pretreatment§–no normal	−0.93	0.065	5561	138
Exponential				
Preoperative*–first normal†	−0.98	0.0009	9.090	0.1000
Preoperative–no normal‡	−0.97	0.0052	9.288	0.1155
Pretreatment§–first normal	−0.97	0.0072	9.067	0.0995
Pretreatment–no normal	−0.96	0.0345	9.427	0.1198

*Includes a preoperative CA 125 level in the regression analysis.

†Includes all postoperative CA 125 levels up to and including the first level <35 U/ml.

‡Includes all postoperative levels of CA 125 up to, but excluding, the first level <35 U/ml.

§The first data point is postoperative in this analysis.

patient underwent biopsy only, with the vast majority of tumor left in situ, time 0 was the first day of chemotherapy. CA 125 levels in serum drawn preoperatively were assigned to day 0 in the absence of serum drawn on the day of surgery. Thirty-two patients had preoperative CA 125 levels measured. For the rest the first determination was after surgery and before the first dose of chemotherapy, which was begun on mean postoperative day 19 (range 4 to 42). We performed regression analysis of the data to evaluate both linear and exponential decay patterns. Most patients did not have CA 125 levels measured every few days after the initiation of therapy. Therefore we tested the effects of omitting some data points in a known setting where complete CA 125 data were available. The four possible combinations evaluated were: (1) preoperative CA 125 available, with data plotted to include all postoperative levels up to and including the first normal CA 125 level, ≤ 35 U/ml; (2) preoperative CA 125 level and only elevated postoperative CA 125 levels (>35 U/ml); (3) postoperative CA 125 levels only, to the first normal level; (4) only elevated postoperative CA 125 levels. The results of this analysis for a typical patient are shown in Table II. The models tested were:

$$\text{Serum CA 125 level} = i - s\ (\text{days after surgery})\ (\text{linear regression}) \tag{1}$$

$$\text{Serum CA 125 level} = \text{EXP}(i - s\ [\text{days after surgery}])\ (\text{exponential regression}) \tag{2}$$

where EXP $= 2.718$, i is the y-axis intercept, which reflects initial tumor burden of CA 125–secreting cells, and s is the slope of the regression curve, which is representative of the rate of disappearance of CA 125 from the serum. This rate reflects the composite effects of surgical cytoreduction and effective chemotherapy. When data are plotted, it is more convenient first to perform a natural logarithmic transformation on equation 2, which gives equation 3:

$$\ln\ (\text{serum CA 125}) = i - s\ (\text{days after surgery}) \tag{3}$$

Equations 2 and 3 are mathematically equivalent.

Table II shows that for each set of data the correlation coefficient r is the highest for the exponential model. When r approaches 1.0, a linear relationship exists; the lower the r value, the looser the relationship. When an analysis of variance of the regression curve is carried out as determined by the F ratio test, the exponential model gives p values with a higher significance than the corresponding linear model. In each case the best-fit, lowest p value is represented by the data where preoperative levels of CA 125 are included in an analysis that proceeds through the first normal level of CA 125 ($r = 0.98$, $p < 0.0009$). However, by virtue of the high r and low p values for each of the four combinations of data points, we conclude that the exponential model can be used to approximate s and i even if we are unsure of the preoperative level of CA 125 or precisely when the CA 125 level normalized.

The CA 125 regression curve for a typical patient who responds completely to therapy is represented by the solid line in Fig. 1. In this case $r = -0.98$ and $p < 0.0009$. This plot results in a straight line. When the combination of surgery and chemotherapy is ineffective, as for the patient whose CA 125 data is plotted as the dashed line in Fig. 1, $r = -0.09$ implies a poor fit of the model. The F ratio approaches 0 and p is much greater than 0.05 ($p = 0.86$) for the best-fit regression line. This analysis was carried out for all of the 64 patients. It was possible to calculate s within 30 to 60 days of surgery. In 12 patients serum CA 125 levels never normalized, and clinical progression of disease occurred. For these patients a regression curve that used all data points up to the first increase in serum CA 125 was generated. The mean number of data points for all patients was 4.5 (range 2 to 10). For 54 patients, three or more measurements of CA 125 were available. For 10 patients only two data points were

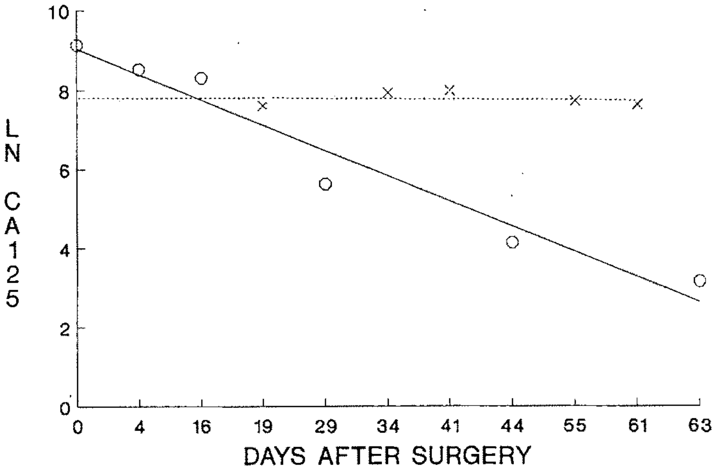

Fig. 1. Exponential regression curves. Comparison of CA 125 regression for responsive cancer (o——o) and unresponsive cancer (x – – – x). Normal level of CA 125 is 35 U/ml (LN 35 = 3.56). *LN,* Natural logarithm.

available. If these patients are excluded, the mean r value for the regression curves was -0.94 indicating that the data fit the model quite well.

Tumor grade, tumor stage (according to International Federation of Gynecology and Obstetrics), and the extent of cytoreductive surgery are several parameters that can influence survival of the patient with ovarian cancer. To develop our model more completely, we investigated the influence of these factors on the regression parameters i and s. These data appear as Table III.

Residual disease. Residual disease was quantitated as none, largest nodule >2 cm, or in between these two parameters. Patients whose tumor was not resected were distinguished from the groups with residual disease and analyzed separately. The values of s and i were calculated for these data, which were stratified on the basis of residual disease, and are presented in Table III. This table shows the trend for more rapid regression of CA 125 as the volume of residual disease decreases from the biopsy-only cases where s is 0.0331 to a value of 0.0835 for the cases with no residual disease. The difference between the two extreme values of s is significant at a p level of 0.015. The difference between the rate of regression for biopsy-only cases and residual disease of <2 cm is also significant ($p < 0.015$). No other pairwise comparisons of s reached significance. The values of i reflect the initial tumor burden. The i values were not statistically different between groups.

The group of patients from Table III who underwent complete resection of ovarian cancer also provides us with the data necessary to calculate the serum half-life of CA 125. When this group of patients is considered as a whole, the mean serum half-life of CA 125 is calculated to be 10.8 ± 5.5 days (range 4 to 21 days).

Stage of disease. Clinical experience dictates that tumor burden generally increases with the stage of disease. We tested our model on this point by stratifying patients on the basis of FIGO stage and generated values for i and s. These data are also shown in Table III. The y-axis intercepts, which are reflective of the initial tumor burden, increased from 5.996 to 6.936 to 7.636 as stage advanced from II to III to IV. Only the difference in i for stages II and IV reached statistical significance ($p < 0.04$). When the values of s were compared pairwise between stages, again only the difference between stage II and stage IV was significant ($p < 0.05$). This result is consistent with the fact that all of the patients with stage II had no residual disease, while the patients with stage IV usually had bulky residual disease.

Table III. Factors influencing regression parameters i and s

Factor	*No. of patients*	*s (mean ± SD)*	*i (mean ± SD)*
Residual disease			
None	13	0.0835 ± 0.0473	6.566 ± 1.956
<2 cm	18	0.0471 ± 0.0313	6.494 ± 1.629
≥2 cm	26	0.0526 ± 0.0486	7.343 ± 1.411
All*	7	0.0331 ± 0.0165	7.373 ± 2.171
FIGO stage			
II	8	0.0814 ± 0.0469	5.996 ± 0.725
III	44	0.0533 ± 0.0451	6.936 ± 1.693
IV	12	0.0446 ± 0.0295	7.636 ± 1.912
Tumor grade			
1	4	0.0275 ± 0.0077	5.788 ± 0.808
2	21	0.0561 ± 0.0436	7.423 ± 1.397
3	39	0.0576 ± 0.0452	6.814 ± 1.837

*Laparotomy performed with biopsy; cytoreductive surgery not done.

Tumor grade. Table III shows the results of application of the model when the patients were stratified

Table IV. Clinical correlation of regression parameters i and s

Clinical course	*No. of patients*	s *(mean ± SD)*	i *(mean ± SD)*
Progression	13	0.0250 ± 0.0240	7.3148 ± 1.594
Recurrence	31	0.0497 ± 0.0326	6.7097 ± 1.504
Disease-free	20	0.0783 ± 0.0475	7.0837 ± 2.024

Table V. Chemotherapy effects

Regimen	*No. of patients*	s *(mean ± SD)*	i *(mean ± SD)*
CAP	6	0.0320 ± 0.0186	6.826 ± 2.439
Low-dose cisplatin	25	0.0380 ± 0.0276	6.542 ± 1.215
High-dose cisplatin	17	0.0671 = 0.0549	7.888 ± 1.546

on the basis of tumor grade. There was a trend toward more rapid response by the more undifferentiated tumors: The value of s increased with increasing grade. The difference in tumor burden was significant only when grade 1 tumors were compared with grade 2 tumors ($p < 0.03$).

Clinical correlation. The clinical relevance of the parameters s and i was determined by grouping the patients according to their response to therapy. Three broad classifications were used: (1) progressive disease during chemotherapy, (2) complete response to therapy on the basis of clinical evaluation and normalization of the serum CA 125 level, followed by recurrence of cancer, and (3) a complete response to therapy with no current evidence of cancer. The mean values of s and i were calculated for these groupings and are displayed in Table IV. This table shows that the tumor burden, as reflected by i, was the same for all groups ($p > 0.24$). On the other hand, there was a steady increase in the rate of decline of the serum CA 125 level, from those with progressive disease ($s = 0.0250$), to those who responded then had recurrence ($s = 0.0497$), to those who remain without disease ($s = 0.0783$). All pairwise comparisons of s were statistically significant ($p < 0.02$). Thus the more rapid the decline in serum CA 125 level, as measured by s, the greater the likelihood of clinical response and cure.

Chemotherapeutic regimen. Finally, we investigated the effects of different chemotherapy regimens. Values of s and i were calculated on the basis of our exponential model. These results appear in Table V. Table V shows that the patients in the high-dose cisplatin group had a higher value of i ($p < 0.04$) only when compared with the value of i for the low-dose cisplatin-treated group. Other pairwise comparisons, e.g., high versus CAP ($p = 0.23$), and low versus CAP ($p = 0.68$), were not significant. Evaluation of treatment response, as measured by the value of s, showed a significant difference for high-dose cisplatin chemotherapy compared with low-dose cisplatin ($p < 0.03$) but not the CAP regimen ($p = 0.14$). Similarly, there was no difference between the CAP regimen when compared with the low-dose cisplatin regimen ($p = 0.62$). For the purpose of these analyses, patients who had complete cytoreduction were removed to prevent bias.

The ideal CA 125 regression curve is generated by monitoring serum levels after complete resection of cancer. For any chemotherapeutic regimen designed, comparison of the rate of CA 125 regression relative to the ideal regression curve provides a measure of its relative effectiveness. The more effective the treatment, the closer the regression line comes to the ideal. Fig. 2 illustrates this point. This figure results from plotting the mean regression curves, which have been normalized for the same tumor burden (i), for complete resection, high-dose cisplatin therapy for residual disease, and low-dose cisplatin therapy for residual disease. When high-dose cisplatinum therapy is used, CA 125 regression approaches that seen for the patient with complete cytoreduction; this value is statistically different from that of low-dose cisplatin therapy ($p < 0.03$).

These data also justify the omission from analysis of patients whose cancers are completely resected when types of chemotherapy are compared. For those patients s approaches the ideal regression curve by virtue of the surgery alone, so that there can be no difference on the basis of chemotherapy over the short term.

Contingency tables were constructed to evaluate the possible influence of tumor grade, extent of residual disease, or FIGO stage. Table VI shows the distribution of covariables for the different chemotherapies. In each instance the calculated value of χ^2 was low and not statistically significant. Thus the different chemotherapy groups were homogeneous from the standpoint of those variables that could have introduced bias.

There was a statistically different mean age between chemotherapy groups. Patients treated with the high-dose cisplatin regimen were slightly younger (mean 59 years) than the group treated with the low-dose cisplatin (mean 66 years) ($p = 0.006$). There was no age difference between the high-dose cisplatin group and the CAP chemotherapy group ($p = 0.25$).

Comment

Most authors[3, 5, 8, 12, 13] agree (1) that for CA 125–positive tumors the level of CA 125 reflects tumor burden and (2) that for a given individual the change in CA 125 level accurately reflects disease status. These observations hold true even when there is no radiologic or physical evidence of residual disease after cytore-

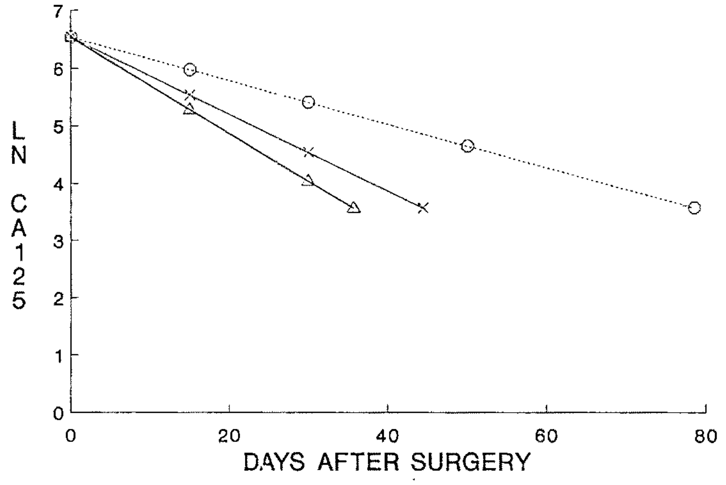

Fig. 2. Cisplatin dose-response curve. Comparison of rate of decline in serum CA 125 levels for patients with residual disease treated with low-dose cisplatin-Cytoxan (o——o) or high-dose cisplatin-Cytoxan (x——x) to ideal regression curve (Δ——Δ) generated from patients with no residual disease. LN (35) = 3.56, which is considered the normal level of CA 125. *LN,* Natural logarithm.

Table VI. Stratification of covariables between modes of chemotherapy

	Chemotherapy		
Covariable	*CAP*	*Low-dose cisplatin*	*High-dose cisplatin*
Residual disease			
None	2	5	4
<2 cm	1	10	4
≥2 cm	1	14	12
All	4	1	1
FIGO stage			
II	1	5	2
III	6	20	14
IV	1	5	5
Grade			
1	1	2	1
2	3	10	8
3	4	18	12
Age of patients (yr, mean ± SD)	59 ± 7	66 ± 9	61 ± 10

ductive surgery. That is to say, a rising CA 125 level correlates well with disease progression and a falling CA 125 level accurately reflects the combined effects of surgery and or effective chemotherapy. This formula provides a model to measure the rate of change of serum CA 125 level as a function of initial tumor burden and subsequent response to treatment:

$$\text{Serum CA 125 level} = \text{EXP}(i - s\ [\text{days after surgery}])$$

The value of s can be calculated within 30 to 60 days of initial surgery for the patient who is started promptly on a regimen of chemotherapy. The value of s reflects the composite effects of surgery and effective chemotherapy. For most patients it is not possible to separately quantitate the relative effects of surgery alone versus those of chemotherapy alone. However, we can approximate these relative effects with the use of data that have been stratified on the basis of residual disease from Table III. The seven patients who had biopsy only and did not undergo cytoreductive surgery are essentially a group treated by primary chemotherapy. The 13 patients who had no residual disease after surgery are our closest approximation to surgery alone even though they also received chemotherapy. The difference in s between these groups was significant at the $p < 0.02$ level, which indicates that cytoreductive surgery is more effective than chemotherapy in producing an initial therapeutic response. This result lends support to the approach of most gynecologic oncologists, who reoperate on patients with ovarian cancer who are referred after an open-and-close operation at an outside institution.[14-17]

The mean serum half-life of 10.8 days that we report is larger than the 4.8-day half-life reported by Canney et al.,[1] who used data from a single patient with completely resected early disease to determine half-life. The difference is explained by the fact that our data base included patients with stage III and IV disease who undoubtedly had residual microscopic foci of cancer that were favorably influenced by chemotherapy. The half-life of CA 125 in other patients also was as short as 4 days even though they had residual macroscopic disease. In these cases the residual disease was either extremely sensitive to chemotherapy or not responsible for secreting CA 125 into the serum. Thus we prefer to report the mean serum half-life and to use it, rather than the minimum half-life value of 4 days, to calculate the ideal regression curve.

Most patients did not have CA 125 levels measured every few days after the initiation of therapy. Before this retrospective study, our standard was to check CA 125 levels immediately before each cycle of chemotherapy. Careful evaluation of the data in Table II indicates that the unavailability of data points from the preoperative time inclusively through normalization of the CA 125 levels does not seriously affect our calculated values of s and i. For the incomplete data sets, the model was still useful since the r values ranged from -0.98 to -0.96 and p ranged from 0.0009 to 0.03345. Our standard now is to measure serum CA 125 before surgery, several days after surgery, on the first day of chemotherapy, and weekly during the initial 6 weeks of chemotherapy to try to refine calculated values of s.

The principle of generating a regression curve is not new. Schlaerth et al.[18] applied this concept to gestational trophoblastic neoplasia in 1981, thereby identifying patients who would require chemotherapy on the basis of departure from the normal regression curve of β-human chorionic gonadotropin. Our study characterizes regression curves for the coelomic epithelial antigen CA 125 in epithelial ovarian cancers and also suggests that clinical outcome can be predicted reliably on the basis of these curves. By logical extension, regression curves that use other tumor markers such as NB/70K, CA 19-9, lipid-associated sialic acid, etc., could be described similarly for those tumors that elaborate these tumor-associated antigens. Furthermore, we suggest that these regression curves or a multivariate regression curve for a spectrum of tumor-associated antigens might predict clinical outcome even more precisely.

Studies have shown that ovarian epithelial carcinomas have heterogeneous immunohistochemical staining properties for CA 125.[13] We do not have evidence at this time that the chemotherapeutic response of the CA 125–secreting cells is the same as that of those that do not secrete CA 125. If our model is to accurately describe effective treatment of epithelial ovarian cancer, this factor forms a critical assumption. Further justification of this assumption can be generated by studying simultaneous markers, which are secreted by different cell types, as well as by using second-assessment celiotomy and disease-free survival as additional end points. These analyses will form the basis of a subsequent report.

Our model also assumes that there is a relationship between the amount of residual tumor after cytoreductive surgery and the serum CA 125 level. It is true that ascites[19] and other confounding variables such as tumor grade, histologic type, and heterogeneity may influence the serum level of CA 125; however, these parameters will be constant for a given patient. When mean pooled values of s for different treatment regimens are compared, it is thus critical to have enough heterogeneity between cohorts so that one covariable does not skew interpretation of the data.

Within biologic systems, dose-response curves generally are sigmoidal in shape.[20] In humans, it is unclear whether treatment with 50, 75, or ≥ 100 mg/m^2 of cisplatin per cycle reaches the plateau region of a typical dose-response curve or merely moves along the linear region of the curve. Ozols et al.[21] and others[22-24] have inferential evidence that taking cisplatin doses to toxic levels may favorably affect survival. When traditional methods of survival analysis are used, the studies necessary to prove this point may take years to mature. The model we have developed provides an eloquently simple means of addressing such clinically relevant issues in a timely fashion. We suggest that various treatment regimens be compared on the basis of the value of s relative to the ideal regression curve, as we have done by comparing low-dose cisplatin chemotherapy with high-dose cisplatin chemotherapy in Fig. 2. While our study groups were homogeneous with regard to surgical stage, grade, and extent of resection, there was a statistically younger patient population treated with the high-dose cisplatin regimen than with the low-dose regimen. Even though 36% of our patients were treated on a randomized basis, there was probably a tendency to treat the older patients whose treatment was not randomly assigned with a "less toxic regimen" in the 1986 to 1988 era. In general, there is a greater tendency for recurrence of disease in older patients[25] so that there could be an age bias influence of our results. On the other hand, there was also a statistically larger tumor burden ($p < 0.04$) for the high-dose regimen than for the low-dose regimen. Thus, since these are potentially offsetting influences, we must have additional patient accrual to solidify the evidence that the high-dose cisplatin regimen is more effective than the low-dose regimen.

In conclusion, the decline in serum CA 125 levels in patients with effectively treated epithelial ovarian cancer follows an exponential regression. These regression curves may be used to generate in vivo dose-response curves and to compare chemotherapeutic regimens. Strong divergence from the ideal regression curve uniformly results in treatment failure. Divergence can be determined within 30 to 60 days of initial surgery. When it occurs, we must begin trials of alternative chemotherapy that is designed to overcome drug resistance earlier than has been the case traditionally. The time has come to utilize the full weight of CA 125 data to make treatment decisions rather than to wait for the findings at second look or the reappearance of bulky disease.

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Uterine rupture at term pregnancy with the use of intracervical prostaglandin E_2 gel for induction of labor

Ron Maymon, MD, Adrian Shulman, MD, Meir Pomeranz, MD, Michael Holtzinger, MD, Luis Haimovich, MD, and Charles Bahary, MD

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Prostaglandin E_2 is a powerful oxytocic agent that reliably initiates labor, even in the presence of an unripe cervix. The low incidence of fetomaternal complication contributes to its universal use. We report a rare case of uterine rupture after intracervical application of prostaglandin E_2 gel. Thus far no prostaglandin compound or method of administration seems to be exempt from such a complication. (AM J OBSTET GYNECOL 1991;165:368-70.)

Key words: Prostaglandins, term pregnancy, uterine rupture

Numerous studies over the past decade have explored the promising results of topical application of prostaglandin E_2 (PGE_2) for cervical ripening or induction of labor without serious maternal or neonatal complications.[1] We present a case of uterine rupture after intracervical application of PGE_2 gel, which to the best of our knowledge is the first reported case of such a catastrophic complication of this agent.

A 28-year-old gravida 3, para 2 woman with two previous uncomplicated deliveries was admitted to the labor ward for induction of labor at 42 weeks' gestation of her third normal pregnancy. Her medical history was noncontributory. At admission the general physical examination and blood clotting studies were within normal limits. Fundal height was appropriate for gesta-

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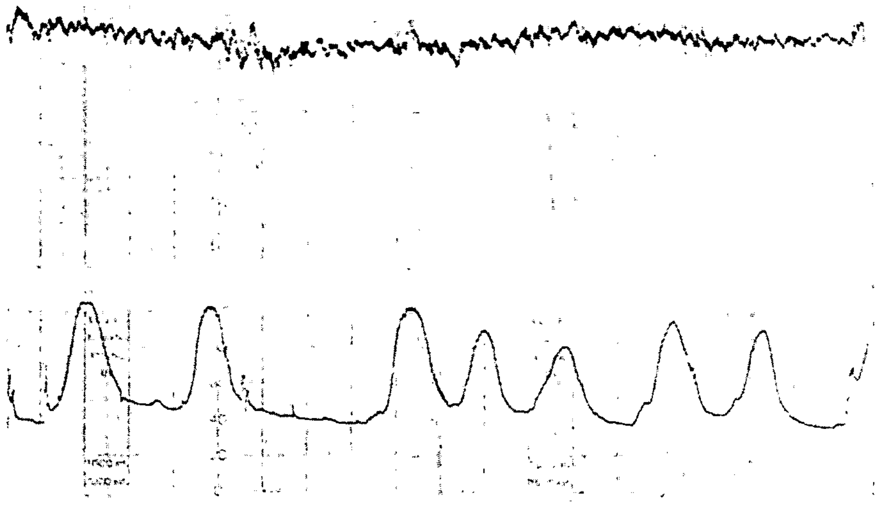

Fig. 1. Monitor tracing shows uterine tachysystole with normal reactive fetal response on external cardiotocographic monitoring.

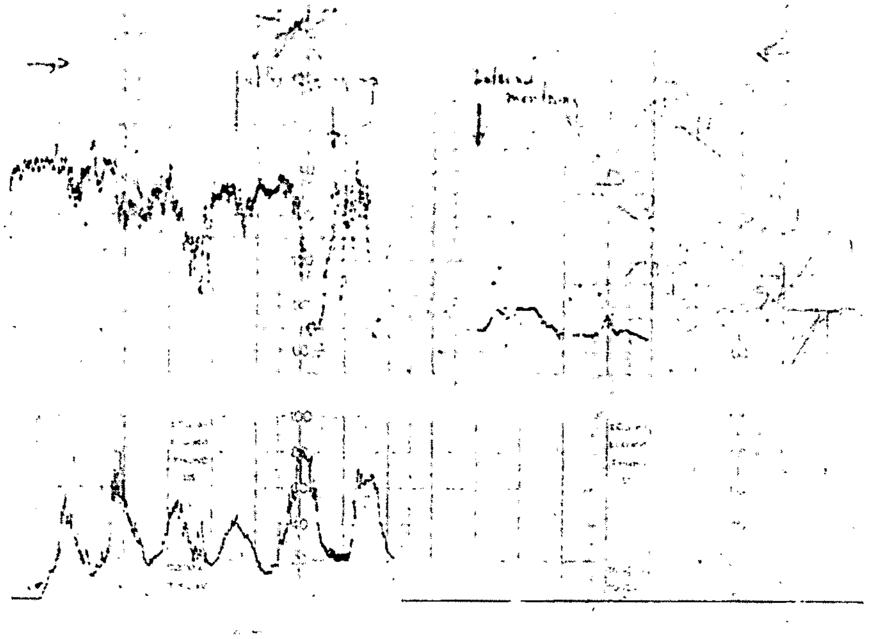

Fig. 2. Monitor tracing shows sudden fetal heart rate decelerations on scalp electrode and uterine tachysystole on external tocographic monitoring.

tional age, and the abdomen was soft with no uterine contractions. On vaginal examination, station of the vertex was −3; the cervix was 20% effaced and 2 cm dilated. The membranes were intact. Cardiotocographic monitoring, performed continuously for 30 minutes, showed normal fetal heart rate with good variability and no uterine contractions. In an attempt to ripen the cervix, the patient was given 0.5 mg PGE_2 gel (Prepidl dinoprostone gel, Upjohn Company) just below the level of the internal cervical os, as previously recommended.[1] One hour later, tachysystolic uterine contractions began and lasted for 20 minutes with no sign of fetal distress (Fig. 1). At this point the patient was given 75 mg meperidine hydrochloride to alleviate the pain and the uterine contractions were ameliorated. Four hours after the PGE_2 application, the cervix was fully dilated, vertex on right occipitotransverse position and the patient was in the lithotomy position when suddenly severe fetal bradycardia was recorded (Fig. 2). A female infant was delivered by rapid vacuum application. The infant weighed 3300 gm and had Apgar scores of 1 and 7 at 1 and 5 minutes, respectively.

Immediately after delivery the mother suddenly displayed signs of severe hemodynamic shock, which in spite of minimal vaginal bleeding, was discovered by pelvic examination to be a result of a ruptured uterus. Emergency laparotomy revealed an 8 cm rupture in the left lateral wall of the uterus, extending from the round ligament close to the internal cervical os. A total abdominal hysterectomy was performed. Postoperative and postnatal recovery was uneventful for both mother and infant.

Comment

Before initiating labor, biochemical changes take place in the connective tissues of the lower uterine segment and cervix. Irrespective of the strength of the uterine contractions, unless these biochemical changes have occurred, the cervix cannot efficiently dilate. Induction of labor with PGE_2 offers the advantages of both induction of further cervical ripening and promotion of myometrial contractility.[1, 2]

According to some authors, detectable myometrial activity is dose-related and more common after intravaginal versus intracervical PGE_2 application.[2] However, signs of myometrial hyperstimulation after intravaginal application of PGE_2 gel have been recorded in 0.6% to 6% of the cases, which is not thought to be more common than that found with oxytocin infusion alone and is virtually nonexistent with a 0.5 mg intracervical dose.[1, 2]

Episodes of fetal heart rate abnormalities and fetal distress, which are associated with uterine hyperstimulation, occur in $<1\%$ of the reported cases, and emergency cesarean sections are rarely necessary.[1]

This case shows that the use of PGE_2 gel at term pregnancy should be used cautiously because individual absorption rates are unpredictable and once absorbed it cannot be discontinued.

In patients who receive PGE$_2$ gel for induction of labor, the obstetrician should be aware of the need for conscientious monitoring, including continuous cardiotocodynomometry, frequent abdominal palpation, and meticulous monitoring of the patient's vital signs for prompt and early diagnosis of uterine rupture. Also, if long-standing hypercontractility occurs, the use of β2 selective sympathomimetic drugs should be considered to reverse this effect. The obstetric dilemma of whether the cervical route for administration of PGE$_2$ gel carries the same risk of uterine rupture as has been reported for other prostaglandin compounds and routes of administration requires further investigation.

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External cephalic version after previous cesarean section

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Approximately 100,000 cesarean sections are performed each year in the United States because of breech presentation. Numerous studies have shown that external cephalic version can eliminate the need for many of these operations. However, because of the fear of uterine rupture, these studies have generally excluded patients who have undergone previous cesarean section. To evaluate the validity of this exclusion policy, we studied patients with one or more previous cesarean sections and breech presentations near term. Version attempts were successful in 82% of 56 patients who had undergone a previous cesarean section. Sixty-five percent of the successful version patients went on to have vaginal birth after cesarean section. There were no serious maternal or fetal complications associated with the version attempts. We conclude that external cephalic version is a reasonable option in patients with prior low transverse cesarean section. (Am J Obstet Gynecol 1991;165:370-2.)

Key words: Vaginal birth after cesarean section, external cephalic version, breech, cesarean section

Many physicians agree that there is an increased risk of adverse perinatal outcome associated with vaginal breech delivery. This has led to an increasing use of cesarean section for infants in breech presentation. At some hospitals the proportion of breech infants delivered by cesarean section is approaching 100%.[1] Several recent studies have demonstrated that the majority of breech presentations can be successfully converted to the normal vertex presentation by the technique of external cephalic version.[2-4] However, these studies have generally excluded patients with prior cesarean section. The purpose of this report is to describe our experience with external cephalic version in 56 patients with prior cesarean sections.

Table I. Comparison of group with prior cesarean section and control group

Parameter	*Prior cesarean section*	*Control*
Number	56	56
Mean gestational age (wk)	37.2	37.4
Mean maternal weight (pound)	167	162
Tocolysis	42	39
Gravidity	3.3	2.1
Parity	1.9	0.7

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Table II. Outcome of external version attempts in group with prior cesarean section and control group

Parameter	*Prior cesarean section (n = 56)* No.	%	*Control (n = 56)* No.	%	*p Value*
Successful version	46	82	34	61	0.02
Reversion to breech	4	7	1	2	NS
Vaginal delivery	30	54	25	45	NS
Birth weight (gm)	3523	—	3498	—	NS
Uterine rupture	0	—	0	—	NS
1-minute Apgar ≤7	5	9	3	5	NS
5-minute Apgar ≤7	0	—	0	—	NS

Table III. External cephalic version and delivery outcomes by indication for prior cesarean section

Indication	*No.*	*Successful version* n	%	*Vaginal delivery* n	%
Breech	23	19	83	11	48
Failure to progress	20	18	90	12	60
Fetal distress	5	4	80	3	60
Transverse Lie	3	2	67	2	67
Other	5	3	60	2	40
TOTAL	56	46		30	

Material and methods

The study was conducted between June 1, 1985, and June 1, 1990, at Kaiser Permanente Medical Centers in Los Angeles, Anaheim, and Riverside. Patients were eligible for inclusion in the study if they were at least 36 weeks' gestation, had a single fetus in breech presentation, and had a history of one or more cesarean sections. Exclusion criteria included ruptured membranes, labor, suspected intrauterine growth retardation, third-trimester bleeding, oligohydramnios, previous classical or vertical uterine scar, or suspicious fetal monitoring pattern on admission. The control group consisted of a series of 56 consecutive external cephalic version patients who met all the above inclusion and exclusion criteria but had no history of prior cesarean section.

The study protocol was approved by the regional institutional review board. Potential risks, benefits, and alternatives to the procedure were explained to each patient and written consent was obtained. Uterine rupture was defined as a separation involving all layers of the uterine wall. Ultrasonographic examination was performed to confirm the breech presentation, assess amniotic fluid volume, and rule out placenta previa. A nonstress test was then performed. At the discretion of the attending physician an initial version attempt was performed without tocolysis. If the version was not readily accomplished or if the fetus was not easily palpated, the patient was treated with either subcutaneous terbutaline sulfate (0.25 mg) or intravenous ritodrine hydrocloride (100 to 300 μg/min). The version techniques used have been previously described in detail.[2-4] The fetal heart rate was monitored during the procedure by real-time ultrasonography. Nonstress testing was repeated immediately after the procedure and again the next day.

Data were entered into a computerized data base. Analysis of data was performed with the χ^2 test or the Fisher exact probability test as appropriate. A p value ≤0.05 was considered to be statistically significant.

Results

Fifty-six patients with prior cesarean section fulfilled the inclusion criteria and underwent attempted external cephalic version. In 46 (82%) of these patients the fetus was successfully rotated to the vertex presentation. Table I compares the demographic characteristics of the study and control groups. With the exception of increased parity in the group with prior cesarean section, both groups were quite similar.

Table II summarizes the outcomes for both groups. Version attempts were more likely to be successful in the group with prior cesarean section (82% vs 61%, $p \leq 0.02$). This finding may simply reflect the increased parity in the group with prior cesarean section. The apparent trend toward increased reversion to breech in the group with prior cesarean section did not reach statistical significance (7% vs 2%, $p \leq 0.36$).

Table III summarizes the outcome of the group with prior cesarean section according to the indication for primary cesarean section. It was interesting to note that

41% of the prior cesarean sections had been performed because of breech presentation.

There were no uterine ruptures in the study or control groups at the time of version attempt or during subsequent trial of labor. Fetal heart rate abnormalities that necessitated cesarean section occurred during attempted external cephalic version in one patient in each group (1.8%). There were no major complications related to any of the version attempts. Four patients had two prior cesarean sections. Four patients had a history of a previous vaginal birth after cesarean section. Two patients had a history of successful external cephalic version in a prior pregnancy.

Comment

In a review article[5] it was concluded that external cephalic version is absolutely contraindicated in patients with previous uterine scars. There is little objective evidence to support this conclusion. The obvious concern is that the manipulations used during attempted version could rupture a previously scarred uterus. We did not find this to be the case. In this study there were no uterine ruptures in 56 version attempts. Because of the limited sample size, this study does not prove that the scarred uterus is at no increased risk of rupture during attempts at external cephalic version. This study indicates that the likelihood of successful external cephalic version is not lowered by the presence of a prior uterine scar.

The documented association of prior cesarean section and placenta previa accreta might generate some concern regarding the risks of external cephalic version in patients with prior cesarean section and anterior placental location. In this study, 39% of the group with prior cesarean section had anterior placental location and all underwent attempted external cephalic version without complications. Clearly, patients with ultrasonographic evidence of placenta previa would not be candidates for attempted external cephalic version regardless of the presence or absence of a prior uterine scar.

If a woman with a history of previous cesarean section presents near term with a breech presentation there are only three alternatives to external cephalic version:

1. Vaginal breech birth. Two recent studies[6, 7] have described good outcomes for vaginal birth after cesarean section with breech presentation in selected cases. However, this alternative will not be acceptable to the growing number of physicians who no longer perform any vaginal breech deliveries. National statistics indicate that the percentage of breech infants delivered by cesarean section rose from 14% in 1970 to 86% in 1986.[1]

2. Expectant management (hoping for spontaneous version). Because the rate of spontaneous version from breech to vertex after 37 weeks' gestation is only about 12%, the prudence of expectant management is questionable.[2]

3. Elective repeat cesarean section. It has been shown that cesarean delivery does not guarantee a good perinatal outcome for patients with breech presentation.[8] The mechanisms of breech delivery can present difficulties even through an abdominal incision.[9]

This study indicates that external cephalic version appears to be an acceptable alternative to elective repeat cesarean section or vaginal breech delivery for properly selected patients with breech presentation near term who have undergone prior cesarean section.

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One-blade rotation of a persistent posterior vertex

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Fifteen women in labor at term with no evidence of protraction or arrest disorder successfully underwent a single-blade rotation of the fetus from occipitoposterior to occipitoanterior position. There were no failures, no fetal injuries, and no significant maternal trauma. This method of forceps rotation appears to be a safe alternative to current methods of managing occipitoposterior positions. (Am J Obstet Gynecol 1991; 165:373-4.)

Key words: Forceps, malposition

Abnormalities of fetal positioning may be associated with dystocia. Therefore a method to assist rotation to the occipitoanterior position may be helpful. This article describes a technique of single-blade rotation that is similar to a method first reported in 1954.

Material and methods

Fifteen patients in spontaneous labor at term with no evidence of protraction or arrest disorder underwent rotation from occipitoposterior or oblique to occipitoanterior with a single pseudofenestrated Tucker-McLane blade. All patients met the standard prerequisites for forceps operation including appropriate application and indication, empty bladder, full cervical dilatation, ruptured membranes, and operator certainty of fetal position and station. All stations at the time of rotation were +3 or +2 with the exception of one patient at +1. The clinical characteristics of the patients and newborns are shown in Table I, and the method of application and rotation is illustrated in Figs. 1 to 4.

Table I. Clinical characteristics ($N = 15$)

Gravidity	3.06 ± 2.41
Parity	1.26 ± 1.44
Rotation to delivery (min)	9.8 ± 11.20
Birth weight (gm)	3643 ± 622.9
Apgar score	
1 min	8.6 ± 0.48
5 min	9.8 ± 0.41

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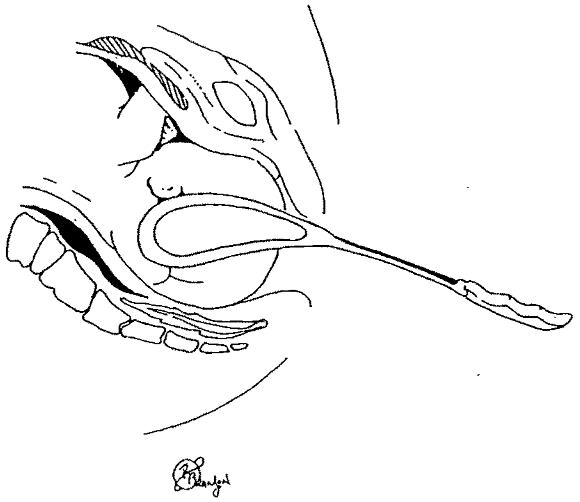

Fig. 1. Blade being introduced upside down to opposite side of maternal pelvis.

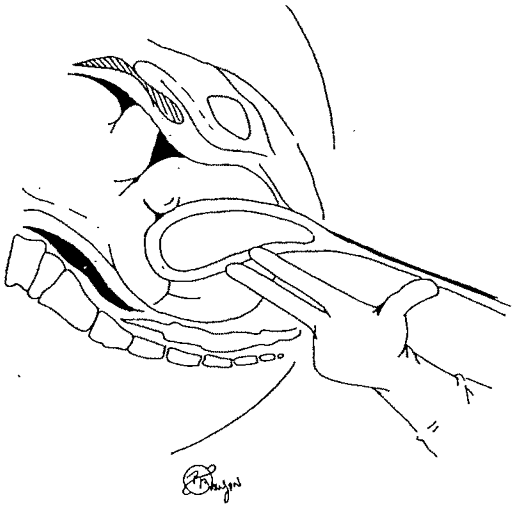

Fig. 2. With second and third fingers, gently rotate up and around under pubic symphysis and "wander" blade over ear (see Fig. 3). Cephalic curve of blade holds malar imminence and convex parietal bone, resulting in rotation of head with blade. If head does not easily rotate, effort is terminated. Care is taken not to angle edge of blade into fetal head or maternal tissue.

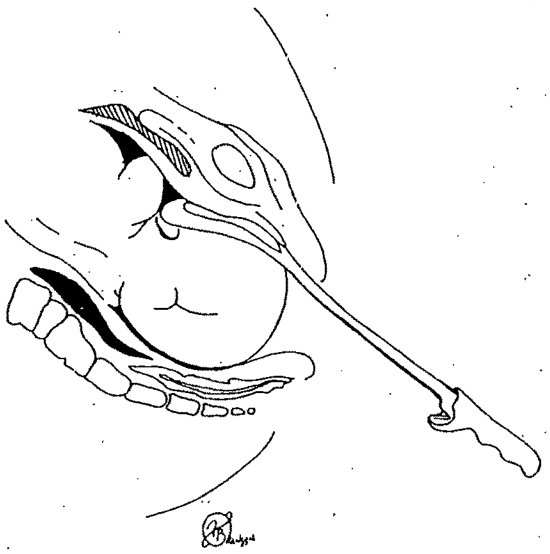

Fig. 3. Blade under symphysis. Cephalic pole is rotating.

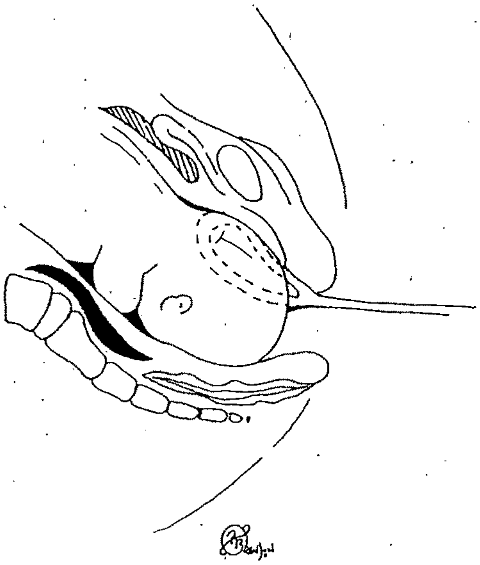

Fig. 4. Presenting part has rotated out of occipitoposterior position. After successful rotation, blade is held in place through several contractions to ensure that fetus does not revert back to occipitoposterior position.

Results

Spontaneous vaginal delivery occurred in 12 patients, and outlet forceps were required in three patients whose fetal heart rate tracing became nonreassuring after the rotation. No fetal injuries or sequelae were noted. Maternal injuries included two fourth-degree lacerations and one slight extension of the episiotomy. All infants were discharged from the hospital with their mothers by 72 hours of life.

Comment

Several management options for occipitoposterior or oblique position are possible. The simplest nonoperative options are watchful expectancy and maternal repositioning. Operative options include manual rotation, delivery of the infant in an occipitoposterior position by forceps or vacuum, or forceps rotation. A number of variations of the two-blade forceps rotation method exist. However, because of the large amount of blade manipulation that is required during these rotations, great skill is necessary to avoid significant maternal-fetal trauma. In an attempt to reduce the trauma associated with forceps rotations, two methods of single-blade rotation were introduced in the 1950s. Maughan[1] used a single fenestrated blade to catch the most anterior fetal ear between the fenestrations and rotate the head out of the posterior position. The second method is the Merchan technique[2]; it is similar to the technique described in this report. With this method the risk of tissue injury should be decreased because blade manipulation is minimal. In summary, in the fetus with an occipitoposterior or oblique position, with no contraindications for forceps operation and a clear indication for expedited delivery, the single-blade rotation appears to be a safe and simple option.

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Comparative efficacy of clindamycin versus erythromycin in eradication of antenatal *Chlamydia trachomatis*

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Antenatal *Chlamydia trachomatis* infections are associated with both maternal and neonatal morbidity. Erythromycin, the only drug recommended for treatment during pregnancy, is often poorly tolerated, thus preventing successful cure. We have done a prospective, randomized, double-blind, placebo-controlled trial to compare the efficacy of clindamycin with that of erythromycin base in eradication of antenatal chlamydia. A total of 126 patients with documented cervical infection were enrolled before 24 weeks' gestation to receive clindamycin (450 mg), erythromycin (333 mg), or placebo orally four times daily for 14 days. Partners received doxycycline, 100 mg, twice daily for 7 days. Both clindamycin and erythromycin were effective agents with cure rates of 92.7% and 83.8%, respectively. Erythromycin therapy was associated with significantly more gastrointestinal complaints than was placebo therapy (23.1% (9/39) vs. 2.4% (1/41), $p < 0.02$) whereas clindamycin was not. Patients who experienced side effects were more likely to be poorly compliant ($p < 0.03$) and patients with moderate-to-good compliance were more likely to be cured than were women who were poorly compliant ($p < 0.002$). Results of test of cure cultures performed immediately on completion of therapy did not differ significantly from those taken 4 weeks later. (AM J OBSTET GYNECOL 1991;165:375-81.)

Key words: *Chlamydia trachomatis,* antenatal antibiotic therapy, clindamycin

Chlamydia trachomatis is the most prevalent sexually transmitted pathogen in many obstetric populations. Although obtaining routine antenatal samples for *C. trachomatis* screening has not been standard in this country, there are several reasons to advocate adoption of this policy. In any given population, *C. trachomatis* is encountered three to five times more frequently than *Neisseria gonorrhoeae*, a pathogen routinely screened for in most obstetric clinics. Recent advances in diagnostic methods have made *C. trachomatis* diagnosis readily available to physicians. Left untreated, a woman may: (1) transmit the organism to her offspring at delivery (60% to 70% of neonates delivered through an infected birth canal will become infected, potentially developing conjunctivitis, pneumonia, otitis media, or gastroenteritis); (2) undergo development of endometritis-salpingitis postpartum (upper genital tract disease will develop in approximately 22% to 34% of women with cervical infection within 6 weeks of delivery[1, 2]); (3) experience an adverse obstetric outcome (in a previous case-control study[3] we found that cervical infection with *C. trachomatis* was significantly associated with preterm premature rupture of the membranes [PROM]); and (4) serve as a vector for continued spread of the disease in the community.

Currently, the only drug recommended for treatment of chlamydial infections during pregnancy is erythromycin. However, some patients are unable to complete a therapeutic course because of undesirable side effects, specifically nausea and gastrointestinal distress. Pregnancy predisposes the patient to development of these complaints, and compliance is frequently poor. In hospitalized patients unable to take oral medications, erythromycin is poorly tolerated when administered intravenously because it is irritating to veins. Alternative therapy with equivalent efficacy but fewer side effects is needed. Preliminary studies with clindamycin in nonpregnant populations suggest that this medication is well tolerated and efficacious.[4, 5] This study was undertaken to determine the comparative efficacy of clindamycin and erythromycin in eradication of *C. trachomatis* from the lower genital tract in pregnant women and whether clindamycin is better tolerated and, hence, promotes patient compliance and cure rates superior to erythromycin.

Material and methods

After approval from our institutional review board and the Federal Food and Drug Administration, a randomized, double-blind, placebo-controlled trial was conducted, enrolling patients from the University of Maryland Hospital Obstetrical Clinic between October 1985 and April 1988. Women with a cervical specimen

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Table I. Demographic characteristics at enrollment

	Treatment group		
	Clindamycin (n = 42)	*Erythromycin (n = 40)*	*Placebo (n = 44)*
Age (yr)	20.3 ± 3.2	21.7 ± 4.2	21.3 ± 4.0
Race (% black)	93	97	91
Unmarried (%)	91	95	91
Insured or employed (% yes)	14	21	24
Gestational age (wk)	19.8 ± 1.8	20.1 ± 2.0	20.1 ± 2.0
Parity*	0.5 ± 0.8	0.6 ± 0.7	0.6 ± 0.8

Data are ± SD.

*Term plus preterm deliveries.

culture-positive for *C. trachomatis* before 24 weeks' gestation were eligible with the following exclusions: sensitivity to study medication; significantly impaired hepatic function; persistent gastrointestinal symptoms or history of colitis; antibiotic therapy after screening and before enrollment; or current treatment with insulin, steroids, carbamazepine, or warfarin.

After informed consent was obtained eligible participants between 16 and 24 weeks' gestation were randomly assigned to one of three treatment groups: (1) erythromycin base tablets (333 mg) and clindamycin placebo, (2) clindamycin capsules (450 mg) and erythromycin placebo, or (3) erythromycin placebo and clindamycin placebo. Group 3, the placebo-control group, resulted from the participation of these patients in a separate investigation to determine whether antenatal treatment of chlamydia improves pregnancy outcome, which required a placebo arm. All patients understood the risks associated with untreated infection. All medications were administered orally, four times a day for 14 days. Partners received doxycycline (100 mg) twice daily for 7 days. Medications were dispensed in blister packs with each dose packaged sequentially in an individual cell, permitting accurate determination of the specific number and timing of missed doses.

Participants were screened for *N. gonorrhoeae* before enrollment and, when indicated, treated with intramuscular aqueous procaine penicillin G and oral probenecid according to guidelines of the Centers for Disease Control. Patients were proscribed for the duration of the pregnancy from receiving additional antibiotics without the approval of the investigator. Infectious conditions that required therapy were treated with antibiotics that do not provide *C. trachomatis* coverage (e.g., nitrofurantoin for urinary tract infections). Women who received antibiotics that might influence *C. trachomatis* recovery were withdrawn from the study.

The initial test of cure for *C. trachomatis* was performed at completion of therapy, 14 days after the first dose of study medication. A second test of cure was obtained approximately 4 weeks later. On admission to the Labor and Delivery Suite for labor or ruptured membranes (whether preterm or term), a final specimen was submitted for *C. trachomatis* culture. All specimens were obtained from the endocervical canal with plastic-handled, cotton-tipped swabs after the ectocervix had been cleared of secretions. Specimens for *C. trachomatis* isolation were placed immediately in transport media and processed within 12 hours. They were inoculated into cycloheximide-treated McCoy cells in shell vial monolayers, incubated for 40 hours, fixed with methanol and stained with fluorescein-conjugated monoclonal antibody to *C. trachomatis* (Kallestad Laboratories, Austin, Tex.). Inclusions were detected by epifluorescence microscopy.

To prevent inadvertent retreatment outside of the study protocol, culture results were not available to members of the clinic medical staff but were reported by study number only to the investigators. All patients with positive culture results at delivery were treated immediately post partum with doxycycline in conventional doses as were their present partners. All infants were followed-up by the pediatrics staff for 3 months.

Patients returned their used pill packs along with those of their partners at the first test of cure. Compliance was assessed as either good, moderate, or poor. Patients who missed four or fewer of the 56 total doses were defined as demonstrating good compliance. Patients who completed at least 28 doses (equivalent to a minimum of 1 week of therapy) exhibited moderate compliance, whereas missing more than 28 doses constituted poor compliance. Because specimens from partners were generally unavailable to document partner cure, it was presumed that partners had been successfully treated if the returned blister packs indicated that no more than two doses had been missed.

Statistical analysis for categoric data was performed with χ^2 tests and by Fisher's exact tests when categories contained fewer than five subjects.

Results

A total of 135 eligible patients were enrolled in the study. Of these, delivery outcome data were available for 126; nine patients were delivered of infants elsewhere and were lost to follow up. Forty-two women received clindamycin, 40 received erythromycin, and 44 received placebo. Demographic characteristics of the patients in each treatment group are shown in Table I. There were no significant differences between the groups at the time of enrollment with regard to any of these parameters, and there were no differences in the number of sexual partners at enrollment or in the num-

Table II. Posttreatment follow-up cultures

	Patients remaining chlamydia positive					
	Test of cure 1		*Test of cure 2*		*Labor and delivery*	
Group	*No.*	*%*	*No.*	*%*	*No.*	*%*
Clindamycin	2/40	5	2/36	6	4/34	12
Erythromycin	4/34	12	5/32	16	8/38	21
Placebo	30/40	75	30/42	71	17/40	43

ber of untreated sexual partners between treatment and delivery.

Both clindamycin and erythromycin were effective agents in the treatment of antenatal *C. trachomatis* infection. Table II lists the failure rates for each of the treatment regimens at the first and second test of cure and at the time of delivery. With cure defined as only negative cultures at the first or second test of cure, 92.7% of patients who received clindamycin and 83.8% of patients who received erythromycin were cured as shown in Table III. Although therapy failed in twice as many patients with erythromycin as it did with clindamycin, this difference was not significant. For those patients who completed at least 28 doses of medication, 94.3% of patients who received clindamycin and 93.3% of patients who received erythromycin were cured.

Eighty-five percent of the treatment population never had a subsequent positive culture throughout the study. In particular, 88.1% of the patients who received clindamycin and 82.5% of erythromycin-treated patients remained disease-free. Of patients who completed at least 28 doses of medication, 88.9% of patients receiving clindamycin and 87.9% of patients receiving erythromycin had no recurrence of *C. trachomatis* during the entire pregnancy.

Overall, among patients who received antibiotic therapy, the first and second test of cure culture results were concordant in 96%. Of the 67 patients who received antibiotics and were culture-negative at the first test of cure, 61 (91%) had no positive cultures for the remainder of the pregnancy. Of the six patients who were subsequently culture-positive, two were already positive 4 weeks after the completion of therapy; one, if not both, of these women had never been cured initially of her infection inasmuch as she took only one dose of the study medication. In one of the four remaining patients there was documented poor partner compliance. Partner compliance data was unavailable for two women. Thus even if we assume these two men were compliant, of those patients who completed therapy and were initially cured, no more than four (6%) had subsequent unexplained recurrences during the remainder of pregnancy.

Table III. Treatment efficacy

Antibiotic	*No.*	*% Cured patients*	*% Cured of patients taking ≥28 doses*
Clindamycin	41	92.7	94.3
Erythromycin	37	83.8	93.3
Placebo	44	22.7	25.0

Nine of the women who received placebo (20%) had no subsequent positive cultures for the remainder of pregnancy, eight of these nine having three successive negative cultures. Careful review of these patients' charts confirmed that (1) all patients were initially culture-positive, (2) all denied taking any antibiotics other than those administered through the study, either before or subsequent to enrollment, and (3) no mention of additional therapy was documented in the chart. Negative cultures from placebo-treated patients were most common at delivery. Indeed, patients who received placebo were twice as likely to be culture-negative at the time of delivery as earlier in pregnancy. Of 40 women in the placebo group for whom culture results were available, 23 (58%) had negative cultures at delivery.

No major side effects were encountered in either antibiotic treatment group; only one patient had therapy discontinued by the investigators. An extensive, pruritic rash developed in this patient that, subsequent to discontinuation of the study medication (erythromycin), was diagnosed as varicella. Two patients who received clindamycin reported development of a rash but both patients completed the entire course of therapy and no rash was apparent at the first test of cure visit. One patient died of toxic shock syndrome 11 days after she was delivered of an infant at another hospital. More than 4 months had elapsed since the partial completion of her course of clindamycin within the study protocol. Overall, 85% of patients who received clindamycin, 77% of patients who received erythromycin, and 98% of patients who received placebo experienced no side effects.

Erythromycin therapy was associated with significantly more gastrointestinal complaints than was placebo therapy ($p < 0.02$). Vomiting, diarrhea, or abdom-

Table IV. Side effects versus compliance

Compliance	No.	Experienced side effects	
		No.	%
Good			
0-4 doses missed	70	8	11.4
Moderate			
5-28 doses missed	39	4	10.3
Poor			
>28 doses missed	9	4	44.4*

*$p < 0.03$, moderate to good compliance versus poor compliance.

inal pain developed in nine of 39 (23.1%) women compared with one placebo-treated patient. In three instances, therapy failure was directly linked to discontinuation of the study medication by the participant because of side effects. In contrast, 10% (4/40) of patients who received clindamycin complained of gastrointestinal side effects, but all these women were able to complete a sufficient number of doses to cure the infections. Only patients who received erythromycin complained of significant abdominal pain or cramping (4/39). (One patient complained of stomach pain after the first dose of clindamycin.) Diarrhea, defined as more than three loose bowel movements in a 24-hour period, developed in two patients while taking erythromycin. One patient who received clindamycin complained of diarrhea and discontinued therapy without discussing this with the investigator. *Candida vaginitis* was an infrequent finding in all three groups and was not temporally related to treatment.

Patient compliance data were available for 118 of the 126 women. Overall, compliance in this indigent, clinic population was quite good. The great majority (92.4%) of women completed at least 28 doses of therapy; 59% did not miss more than four doses. The development of side effects and patient compliance were interrelated as shown in Table IV. Patients who experienced side effects were more likely to be poorly compliant ($p < 0.03$). In the erythromycin-treatment group, three of the four patients (75%) with poor compliance for whom side effects data were available, experienced side effects compared with 25.0% (4/16) of patients with moderate compliance and 11.8% (2/17) with good compliance. Women treated with placebo were the most compliant whereas patients who received erythromycin were the least compliant, although these differences were not significant ($p = 0.06$) (Fig. 1).

Patient compliance was also related to cure (Table V). Women with moderate-to-good compliance were more likely to be cured than women who were poorly compliant ($p < 0.002$). The difference in cure rates between patients with moderate compliance and those with good compliance was not significant. Patients who completed at least 28 doses of therapy were as likely to be cured as those who completed 52 doses of therapy.

Compliance data were available for 96 of the partners; 89.6% were compliant, but it is possible that many of the partners for whom compliance data were unavailable because of failure to return the pill packs were noncompliant. Several patients did not resume sexual activity with a noncompliant partner after treatment.

Partner compliance was also significantly associated with cure. Ten percent of antibiotic-treated subjects with compliant partners had a subsequent positive chlamydia culture compared with 50% of subjects whose partner compliance was poor and 22% whose partner compliance was unavailable ($p < 0.03$). Even in placebo-treated patients, a partner effect on cure was suggested. In the nine patients with only negative cultures after treatment, partners missed no doses of medication or the patient denied sexual contact after treatment. In contrast, in 11 of 35 (31.4%) placebo-treated patients in whom at least one subsequent culture was positive, posttreatment sexual contact with a noncompliant partner could not be excluded.

Comment

Each year more than 4 million cases of chlamydial infection occur in the United States and 155,000 infants are born to mothers with genital chlamydia.[6] Chlamydial conjunctivitis will develop in approximately 30% to 40% of exposed infants, whereas pneumonia occurs in 10% to 20%.[7] Several investigators have noted an association between maternal cervical infection with *C. trachomatis* and preterm delivery.[3, 8-10] Currently, the only drug recommended for treatment of chlamydial infections during pregnancy is erythromycin.

Erythromycin has demonstrated efficacy in vivo in the treatment of chlamydial cervicitis with reported cures varying between 76% and 100% in nonpregnant populations.[11-13] Little information is available on doses or efficacy in the pregnant patient. Schachter et al.[14] have shown that antenatal treatment with erythromycin can successfully eradicate *C. trachomatis* from the maternal genital tract during pregnancy. Unfortunately, at the recommended dose of 500 mg, orally, four times daily, a significant number of patients experience undesirable gastrointestinal side effects and are unable to complete a therapeutic course. Robson et al.[15] noted that gastrointestinal complaints, including nausea, vomiting, and abdominal pain, occurred in 10 of 19 nonpregnant patients on this regimen, whereas Bowie et al.[16] had to discontinue this arm of their study when among the first 13 patients, major side effects developed in five patients. These side effects necessitated either stoppage of treatment or reduction of the dose by at least half. Reduction of the dose to 250 mg

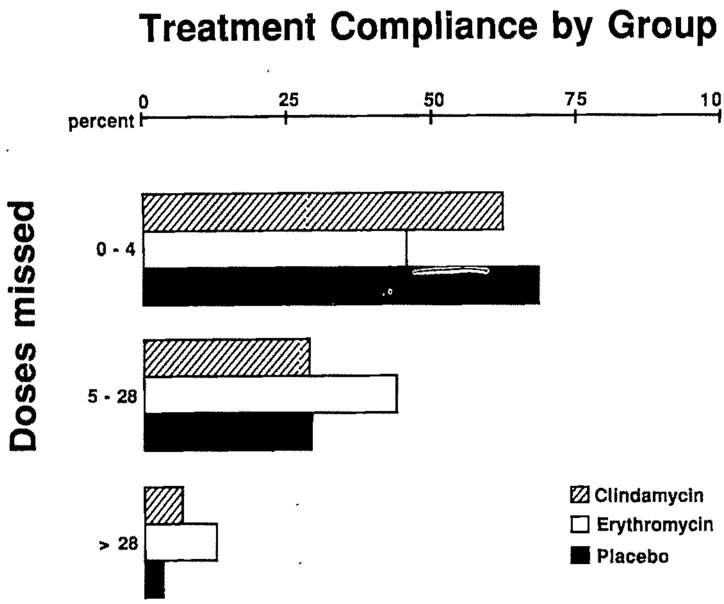

Fig. 1. Percentage of patients in each treatment group with good, moderate, or poor compliance. Similar compliance was exhibited by placebo and clindamycin groups.

orally, four times daily as recommended in the guidelines of the Centers for Disease Control, may not eliminate these side effects and requires extention of the course of therapy for an additional week, which is burdensome to many patients. In addition, relative resistance of *C. trachomatis* to erythromycin has been reported.[17]

Several antibiotics have demonstrated efficacy in the treatment of chlamydial cervicitis in the nonpregnant patient. In addition to erythromycin, tetracycline,[18] doxycycline,[19] sulfisoxazole,[16] and trimethoprim-sulfamethoxazole[18] are effective but are generally considered contraindicated during pregnancy. Even in nonpregnant patients trimethoprim-sulfamethoxazole has been associated with neurologic side effects in 36% of treated women.[18] Clindamycin has been shown to have both in vitro[20] and in vivo[21] activity against strains of *C. trachomatis*. The minimal inhibitory concentration of clindamycin is tenfold greater than that of erythromycin but serum levels remain above the minimal inhibitory concentration for almost 4 hours after a single 450 mg oral dose of clindamycin. Although there is no information on the use of clindamycin to eradicate *C. trachomatis* during pregnancy, there have been no reports of adverse fetal or maternal outcome when clindamycin has been used to treat other conditions during pregnancy.

Our results demonstrate that clindamycin is an effective and well-tolerated antimicrobial agent for the treatment of antenatal cervical chlamydia infection. More than 94% of patients who completed at least 28 doses of therapy were cured. One week of treatment appears to be sufficient and there does not appear to be any advantage to extending therapy beyond 28 doses. When compared with erythromycin, clindamycin was equivalent in efficacy. Although twice as many patients remained infected after treatment with erythromycin as did after receiving clindamycin, this was not statistically significant but did appear to be related to patient compliance. Patients who took similar numbers of doses had similar cure rates with either drug. A larger study would be necessary to exclude the possibility of β-error and the superiority of clindamycin.

With the use of a 333 mg preparation of erythromycin we found cure rates within the range previously reported in the literature for a variety of dosing regimens. However, a significant number (23.1%) of women still experienced gastrointestinal side effects,

Table V. Cure versus compliance in antibiotic-treated patients

	Cured	
Compliance	*No.*	*%*
Good		
0-4 doses missed	41/42	97.6
Moderate		
5-28 doses missed	24/27	88.9
Poor		
>28 doses missed	3/8	37.5*

*$p < 0.00004$, moderate-to-good compliance versus poor compliance.

although not as frequently as reported by other investigators with the 500 mg dose. Vomiting, diarrhea, or abdominal cramps developed in these patients and three of them completed less than 1 week of therapy because of these side effects. Clindamycin was better tolerated and did not require discontinuation of therapy. Rash, the only other side effect, was uncommon and not serious. Pseudomembranous colitis did not occur in the study population.

Not only were both medications effective, but 85% of the study participants did not have a documented recurrence of *C. trachomatis* for the remainder of pregnancy. Most of those initially culture-negative after treatment (91%) had no subsequent positive cultures. This is in contrast with the group B streptococcus, which although reported to be associated with preterm delivery cannot be eliminated from the vagina for more than a few weeks in most cases, making it impractical to treat early in pregnancy as a means to reduce neonatal complications. Patients in this study were not treated before 16 weeks' gestation to avoid the period of fetal organogenesis and greatest incidence of pregnancy-related nausea. However, all patients were treated before 24 weeks' gestation which might be early enough to reduce the incidence of *C. trachomatis*-related prematurity. Additional studies are necessary to establish whether antenatal treatment of *C. trachomatis* will reduce adverse pregnancy outcome.

A surprising number of patients in the placebo group were also "cured" of infections during pregnancy. Eight patients (18%) had three successive negative cultures after the initial positive screening culture, and no evidence of any other form of treatment could be found. It is unlikely that all eight patients had three successive false-negative cultures and therefore, as with group B streptococci and many other pathogens, it may be possible for host defenses alone to eliminate the infection antepartum. This may be particularly likely if there is no further sexual activity with an infected partner.

It has been recommended that test of cure cultures for *C. trachomatis* be obtained 3 to 4 weeks after completion of therapy. The rationale is that because of the extraordinarily long replication cycle of *C. trachomatis*, a long interval between end of therapy and test of cure is necessary to differentiate between true eradication of the organism and mere suppression. However, we found that for 96% of women results of the first test of cure obtained immediately after the completion of therapy were concordant with results of the second test of cure. Although a negative test of cure followed by a positive test of cure may represent evidence of *C. trachomatis* suppression it may equally represent an example of reinfection with the organism. Because patients with a negative second test of cure culture may also become reinfected (although this occurred in only 2.8% of our study population) it apppears that an acceptable and more practical alternative is to obtain a test of cure culture at the first clinic visit after completion of therapy and to obtain a subsequent screening culture later in pregnancy.

Whereas there was no difference in accuracy between the first and second test of cure for women who received placebo, cultures taken in the delivery suite once the patient was in labor were twice as likely to yield negative results as were antepartum cultures. It is possible that these cases represent spontaneous cures; however, it is more likely that adequate sampling is more difficult during active labor when the cervix may be significantly effaced, the fetal head well-applied to the columnar epithelium of the endocervical canal, the membranes ruptured, and when blood, mucus and intraamniotic debris may contaminate the specimen. Studies in which cultures are performed only at delivery may therefore significantly underestimate the role of *C. trachomatis* in peripartum events. In addition to being less accurate, *C. trachomatis* cultures obtained during labor rarely provide results within a time frame that permits the prevention of vertical transmission.

Successful treatment requires patient and partner compliance in addition to an effective drug. Compliance, in turn, is dependent on patient education as to the importance of treatment and the method of treatment, the ready availability of the treatment, and reduction of any adverse consequences related to treatment. The successful results achieved in this study are a result of such a three-pronged approach. Patients were anxious to rid themselves of an organism that was a health hazard to themselves and their infants. They understood that this was possible only if their partners were cured as well. Most important, medication for the patient and her partner was supplied directly to the patient. Requiring the partner to present to a clinic for diagnosis and treatment is a significant impediment to the successful treatment of women. In our clinics this approach has been associated with a 40% failure rate, more than double that of this study.

We conclude that clindamycin is an effective alternative to standard treatment regimens for chlamydia during pregnancy. Clindamycin appears to be particularly well tolerated by patients and may be associated with better compliance than erythromycin. For patients unable to tolerate erythromycin, clindamycin is an excellent alternative, but because of its greater cost larger studies are necessary to confirm improved cure rates before routine use of clindamycin can be advocated.

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Enhanced sensitization after cordocentesis in a rhesus-isoimmunized pregnancy

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It has been suggested that the optical density at 450 nm may be an unreliable predictor of the severity of fetal anemia in the midtrimester of pregnancy; therefore fetal blood sampling, rather than amniotic fluid evaluation, should be performed in all isoimmunized pregnancies with elevated maternal antibody titers in the midtrimester. Potential complications of such an approach are discussed and an alternative plan of management is offered. (AM J OBSTET GYNECOL 1991;165:382-3.)

Key words: Rh isoimmunization, cordocentesis, optical density at 450 nm

Amniotic fluid assessment of the optical density at 450 nm (ΔOD_{450}) has been used as an indicator of the severity of fetal anemia secondary to red blood cell isoimmunization. Recently, Nicolaides et al.[1] have suggested that the ΔOD_{450} may be an unreliable predictor of the severity of fetal anemia in the midtrimester of pregnancy. In contrast, fetal blood sampling by cordocentesis allows direct access to the fetal circulation and precise evaluation of the fetal hematocrit. Therefore they suggested that fetal blood sampling, rather than amniotic fluid evaluation, should be performed in all isoimmunized pregnancies with elevated maternal antibody titers in the midtrimester. There are some controversial issues associated with this approach. For example, the fetus whose antigen status is the same as its mother and who is not at risk for hemolytic anemia would be exposed to possible complications associated with cordocentesis, such as bleeding, cord hematoma, umbilical artery spasm, infection, and death. The following case report addresses another hazard of fetal blood sampling, namely, that it may introduce fetal red blood cells into the maternal circulation and thereby increase maternal antibody production and the severity of the isoimmunization.

Case report

A 25-year-old woman, gravida 4, para 2-1-0-3, had known anti-D isoimmunization. Her second pregnancy resulted in delivery of an Rh-positive infant who required exchange transfusion after delivery. In her subsequent pregnancy, serial amniocentesis and amniotic fluid ΔOD_{450} measurements were performed, the results of which remained reassuring. At delivery, the infant was Rh negative, indicating that the patient's husband was heterozygous for the D antigen. During her fourth pregnancy, the maternal anti-D antibody titer was 1:8 at 24 weeks' gestation, when she was referred to our institution. The patient was offered the option of either amniocentesis and amniotic fluid ΔOD_{450} measurement or cordocentesis for determination of fetal blood type and hematocrit. The patient was informed that cordocentesis may have a higher risk of complications than amniocentesis has; however, it offered the potential for a single procedure if the fetus was Rh negative, whereas serial amniocentesis would be required even if initial results were reassuring. Additionally, cordocentesis could more precisely determine the need for therapy if the fetus was Rh positive. The patient chose to have fetal blood sampling performed because of the potential for a single procedure.

At 25 weeks 3 days of gestation cordocentesis was performed under ultrasonographic guidance. A 22-gauge, 5-inch spinal needle was introduced into the umbilical vein at its placental insertion, traversing the anterior placenta. A single uterine and cord puncture was required for sampling, and the duration of the procedure was <10 minutes. The fetus was Rh positive with positive results of direct Coombs testing at an antibody titer of 1:4. The fetal hematocrit was 31%, and transfusion was not performed. Kleihauer-Betke testing performed on the maternal blood sample after the procedure was negative for fetal red blood cells.

Weekly ultrasonographic examination and maternal antibody evaluation were performed with plans to repeat fetal blood sampling 2 to 4 weeks after the initial procedure. The maternal antibody titer increased to 1:4096 3 weeks after the initial procedure. Ascites and scalp edema were observed on ultrasonographic examination. Repeat fetal blood sampling was performed, and the fetal hematocrit was 12% and the fetal antibody titer was 1:512. Fifty milliliters of irradiated, cytomegalovirus-negative, type O, Rh-negative packed red blood cells were transfused into the umbilical vein, increasing the fetal hematocrit to 38%. Sampling and transfusion were accomplished through a single cord

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puncture and the duration of the procedure was approximately 25 minutes. After the procedure, nonremediable fetal bradycardia occurred, which prompted an emergency cesarean section. A 1505 gm male infant was delivered with an umbilical artery pH of 7.34 and a cord hematocrit of 36%. An umbilical cord hematoma was observed at the site of cord puncture and was presumed to be the cause of the bradycardia.

The infant required a single exchange transfusion during the first 48 hours of life and received three additional simple transfusions. Phototherapy for hyperbilirubinemia was required for 7 days. The infant had intermittent mechanical ventilation for the first 24 hours and oxygen therapy for 3 days because of mild respiratory distress syndrome. He was eventually discharged weighing 2100 gm and doing well on day 40 of life.

Comment

Cordocentesis has improved both evaluation and therapy in fetuses with severe red blood cell isoimmunization. Complications associated with this procedure, such as premature rupture of membranes, premature labor, placental bleeding, and infection, are similar to those observed with amniocentesis. Complications involving the umbilical cord, such as hematoma formation, bleeding, and vasospasm, are unique to this procedure. Additionally, fetomaternal bleeding may occur. Passage of fetal red blood cells into the maternal circulation may stimulate maternal antibody production in isoimmunized pregnancies, potentially increasing the severity of continuing fetal hemolytic anemia. In the case presented, it is not possible to be certain whether fetomaternal bleeding during the initial fetal blood sampling stimulated increased maternal antibody production. However, the maternal antibody titer rise from 1:8 to 1:4096 and the development of severe anemia and hydrops in 3 weeks were dramatic and atypical events.

The amniotic fluid values of ΔOD_{450} are generally a reliable indicator of the severity of fetal isoimmunization during the third trimester of pregnancy. Nicolaides et al.[1] have suggested that amniotic fluid ΔOD_{450} correlated poorly with fetal hematocrit in the second trimester, such that the only reliable method to evaluate the severity of fetal isoimmunization in the second trimester was the direct measurement of fetal hemoglobin or hematocrit. However, analysis of their data shows that all of the severely anemic (hemoglobin, <6 gm/dl) fetuses had ΔOD_{450} values >0.1 in the second trimester. Evaluation of amniotic fluid ΔOD_{450} has shown that measurements rise between 14 and 20 weeks' gestation, plateau between 20 and 26 weeks' gestation, and then trend downward during the third trimester.[2] In addition, the 95th percentile confidence limit of ΔOD_{450} values during the second trimester is only slightly >0.1. Therefore the majority of amniotic fluid sampled during the second trimester would be expected to have ΔOD_{450} values <0.1, which could be used as a cutoff value to determine whether to proceed to cordocentesis.

This case report suggests that there is potential risk of increased maternal antibody production to be considered when fetal hematocrit assessment by cordocentesis is contemplated rather than ΔOD_{450} assessment by amniocentesis in pregnancies complicated by isoimmunization in the second trimester. In the pregnancy with paternal heterozygosity, cordocentesis to determine fetal blood type may offer the opportunity for a single procedure. However, second-trimester amniocentesis for ΔOD_{450} should continue to be considered in the management of these fetuses. Although fetomaternal hemorrhage may occur during amniocentesis, it is probably more likely to occur during cordocentesis with invasion of the fetal circulation. We would propose a management scheme in the second trimester where only those fetuses with ΔOD_{450} values >0.1 undergo fetal blood sampling, thereby reducing the number of cases requiring fetal blood sampling but still indentifying severely sensitized fetuses. Enhanced sensitization after cordocentesis, as well as cord complications, would likely be avoided in the majority of isoimmunized pregnancies.

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Congenital hydronephrosis: Correlation of fetal ultrasonographic findings with infant outcome

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Although congenital hydronephrosis is a common fetal disorder, ultrasonographic criteria for prenatal diagnosis remain poorly defined. In this study prenatal ultrasonographic findings were correlated with postnatal outcome in 63 fetuses with suspected hydronephrosis. Prenatal ultrasonographic measurements included length, anteroposterior diameter, and transverse diameter of the kidney and renal pelvis, as well as dorsal renal parenchymal thickness. In 45 of the 63 fetuses, hydronephrosis was confirmed postnatally. These infants were divided into two groups on the basis of renal status: (1) abnormal renal function and/or surgery required ($n = 31$) and (2) normal renal function with no surgery required ($n = 14$). The anteroposterior diameter of the renal pelvis was the simplest and most sensitive technique for prenatal diagnosis of congenital hydronephrosis, allowing identification of 100% of cases. Postnatal follow-up studies are warranted if an anteroposterior pelvic diameter is ≥4 mm before 33 weeks or ≥7 mm after 33 weeks. (AM J OBSTET GYNECOL 1991;165:384-8.)

Key words: Congenital hydronephrosis, prenatal diagnosis, ultrasonography, infant outcome

Congenital hydronephrosis is a commonly encountered birth defect, occurring with a frequency of 1 in every 330 infants (unpublished data). Clinical presentation is variable and may include a palpable abdominal mass, intermittent pain, vomiting, failure to thrive, or urinary tract infection. Surgical correction of congenital hydronephrosis within 6 to 12 months of birth is typically associated with good outcome whereas delayed diagnosis carries an increased risk of renal impairment.[1, 2] Unfortunately, diagnosis on clinical grounds alone is often delayed with <25% of cases detected by age 1 and only 55% by age 5.[3] Prenatal ultrasonography affords the opportunity for early recognition and treatment of congenital hydronephrosis with hopeful preservation of renal function.

While several ultrasonographic criteria have been suggested for prenatal diagnosis of congenital hydronephrosis,[4-8] few are based on long-term infant outcome and assessment of renal function. The purpose of this investigation was to assess the relative accuracy of these ultrasonographic techniques in the same population to determine the best predictor(s) of renal compromise and/or the need for surgical intervention after birth.

Table I. Congenital hydronephrosis ($N = 45$)

	No.
Associated malformations	6 (9.5%)
Heart defects	3
Pulmonary sequestration	1
Pyloric stenosis	1
Hypospadias	1
Trisomy 21	1

Table II. Congenital hydronephrosis: Site of obstruction

Level of obstruction	*Frequency*	*Ultrasonographic accuracy*
Ureteropelvic junction	67% (40/60)	97% (39/40)
Ureterovesical junction	14% (9/60)	33% (3/9)
Ureterocele	3% (2/60)	50% (1/2)
Other lesions*	14% (9/60)	56% (5/9)

*Isolated reflux, dilated extrarenal pelvis, renal duplication, multicystic renal dysplasia.

Material and methods

The study population was composed of 97 kidneys from 63 fetuses with suspected hydronephrosis on the basis of gross ultrasonographic appearance. All ultrasonographic examinations were conducted in the Washington University Obstetrical Ultrasound Laboratory between 1982 and 1988. Fetuses with bladder outlet obstruction were excluded. A total of 246 ultrasono-

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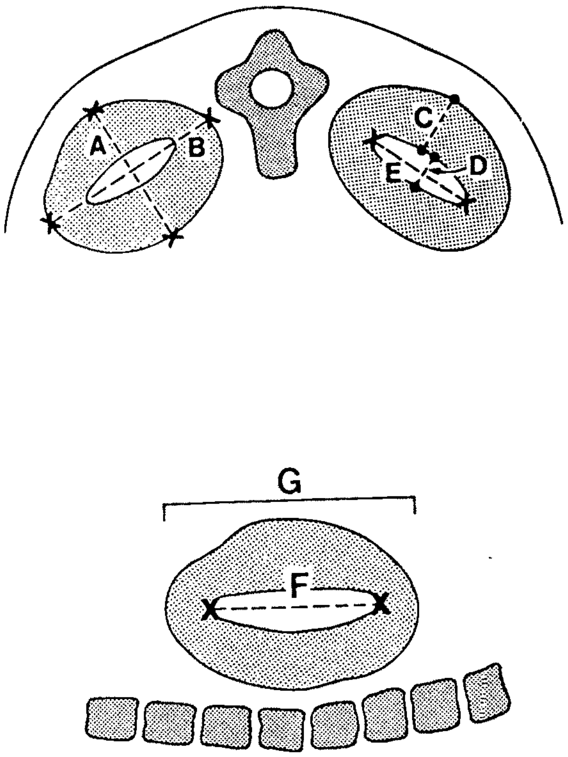

Fig. 1. Technique for ultrasonographic measurement of renal dimensions. *A*, Anteroposterior diameter of kidney; *B*, transverse diameter of kidney; *C*, dorsal parenchymal thickness; *D*, anteroposterior diameter of renal pelvis; *E*, transverse diameter of renal pelvis; *F*, length of renal pelvis; *G*, length of kidney.

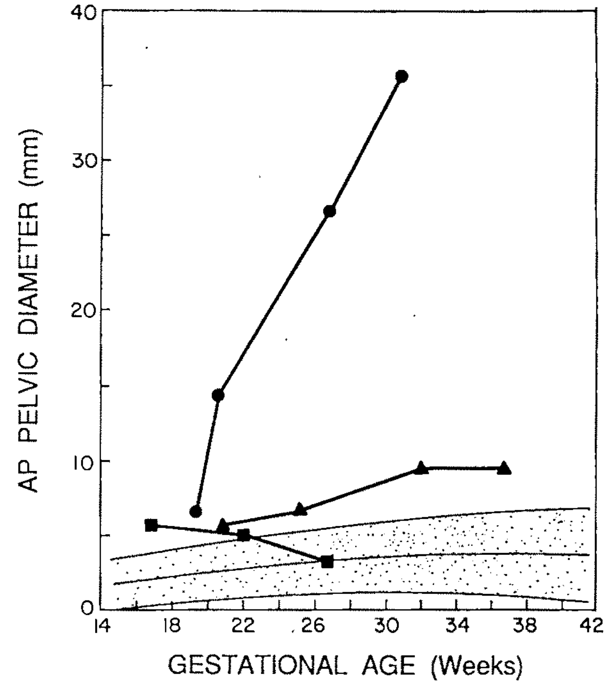

Fig. 2. Natural history of congenital hydronephrosis. Case 39 (●) was classified as having progessive lesion. Fetus in case 32 (▲) had stable hydronephrosis with serial anteroposterior pelvic diameters that paralleled normal reference range. In case 55 (■) spontaneous resolution was observed.

graphic studies were performed for an average of 3.9 per fetus. The examinations were divided into three gestational age time frames (14 to 23.9, 24 to 32.9, and 33 to 42 weeks) with only a single examination per fetus included in each. The following renal measurements were obtained at each ultrasonographic examination: (1) kidney length, transverse diameter, and anteroposterior diameter; (2) renal pelvis length, transverse diameter, and anteroposterior diameter; (3) maximal renal parenchymal thickness.

Kidney and renal pelvis lengths were measured in a coronal plane whereas transverse and anteroposterior dimensions were measured in an axial plane through the fetal abdomen. Renal parenchymal thickness was measured along the dorsum of the kidney when viewed in an axial plane (Fig. 1).

Infant outcome was collected by chart review. The mean duration of follow-up was 20 months with a range of 1 to 71 months. Primary end points included: (1) the accuracy of each ultrasonographic parameter in diagnosing congenital hydronephrosis and (2) the ability of ultrasonography to predict adverse outcome as determined by the presence of renal compromise and/or need for surgical intervention. Postnatal congenital hydronephrosis was confirmed by ultrasonography, intravenous pyelography, or renal scintigraphy. Criteria for defining renal compromise included any of the following findings: (1) serum creatinine level >0.7 mg/dl more than 7 days after delivery; (2) cortical atrophy identified by intravenous pyelography, postnatal sonogram, or renal scintillation scan; (3) differential excretion >20% on renal scintillation scan, or (4) significant urinary tract infection (more than two in female infants and more than one in male infants).

To evaluate which fetal renal dimensions best predict outcome, the following ultrasonographic parameters were examined: (1) anteroposterior diameter of the renal pelvis, (2) ratio of anteroposterior diameters of renal pelvis to kidney, (3) ratio of transverse diameters of the renal pelvis to kidney, (4) kidney circumference/abdominal circumference ratio, and (5) maximal renal parenchymal thickness.

Multiple thresholds were analyzed for each of these parameters and correlated with infant outcome. The goal was to choose the threshold with the highest sensitivity for predicting congenital hydronephrosis to minimize the chance of missing a clinically significant lesion. False-positive rates for each threshold also were determined.

Secondary analyses included the clinical significance of prenatally detected calicectasis, the accuracy of pre-

Table III. Anteroposterior pelvic diameter versus risk of postnatal congenital hydronephrosis

	14-23 wk		*24-32 wk*		*33-42 wk*	
Anteroposterior pelvic diameter (mm)	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*
≥4	100	55	100	42	100	24
≥7	61	35	91	34	100	21
≥10	32	18	74	14	82	18

Table IV. Anteroposterior diameter pelvis/kidney versus risk of postnatal congenital hydronephrosis

	14-22 wk		*23-32 wk*		*33-42 wk*	
Anteroposterior diameter, pelvis/kidney ratio	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*
>0.28	100	50	90	37	91	20
>0.30	92	48	88	33	84	20
>0.40	64	47	69	15	68	21
>0.50	40	47	52	8	50	15

natal ultrasonography in predicting the level of obstruction, the frequency of associated malformation, and the natural history of the obstructive process during fetal life.

Results

The data base consisted of 97 kidneys from 63 fetuses with suspected congenital hydronephrosis on the basis of gross ultrasonographic appearance. In 56% of cases this was an incidental finding during obstetric ultrasonographic examination for other indications. The remaining 44% of fetuses were referred because of a suspected renal anomaly identified during a prior outside ultrasonographic examination.

Congenital hydronephrosis was confirmed by postnatal evaluation in 45 (71%) of the 63 fetuses. Bilateral lesions were present in 15 (33%) of the 45 infants, for a total of 60 index kidneys. Among unilateral cases the left kidney was more commonly involved (57%). As expected, a strong male predilection was found with a male/female ratio of 4.5:1. A single umbilical arery was present in 3.2% of cases. Major nonrenal malformations were identified in 9.5% of infants with congenital heart disease most commonly observed (Table I).

Ureteropelvic junction obstruction was the most common underlying lesion, accounting for nearly two thirds of all cases (Table II). The ureterovesical junction was the next most common site of obstruction (14%), followed by ureterocele (5%) and a variety of other lesions. The accuracy of fetal ultrasonography in predicting the level of obstruction varied widely by underlying lesion. For example, 97% of ureteropelvic junction obstructions were correctly identified versus only 33% of ureterovesical junction obstructions (Table II).

The natural history of congenital hydronephrosis was established by analyzing data from 39 hydronephrotic kidneys monitored with serial ultrasonographic studies. A graph was established for each kidney; the anteroposterior renal pelvic diameter was plotted against gestational age and compared with a similar nomogram derived from a control population of 137 normal fetuses. Congenital hydronephrosis was considered progressive if the observed slope exceeded that of the reference curve (Fig. 2). With this approach, 14 of 39 obstructive lesions (36%) were considered stable throughout gestation whereas progressive obstruction was noted in 37% (14/39) and some improvement in the remaining 28% (11/39). Amniotic fluid volume was normal in 87% (39/45) of hydronephrotic pregnancies whereas oligohydramnios was noted in four pregnancies (9%) and polyhydramnios in two cases (4%).

Six different ultrasonographic parameters were evaluated to determine the best predictor(s) of postnatally confirmed congenital hydronephrosis and related morbidity.

Anteroposterior renal pelvic diameter. In analyzing the data three different thresholds were examined: ≥4, ≥7, and ≥10 mm (Table III). Before 33 weeks' gestation, a threshold of ≥4 mm was required to achieve a sensitivity of 100%. During the last 7 weeks of pregnancy, a threshold of ≥7 mm could be used while still maintaining a sensitivity of 100%.

Anteroposterior diameter of renal pelvis/kidney ratio. Thresholds of >0.28, >0.30, >0.40, and >0.50 were analyzed (Table IV). An anteroposterior pelvis/kidney ratio of >0.28 was required to achieve a sensitivity of 90% to 100%.

Transverse diameter of renal pelvis/kidney ratio. Thresholds of >0.3, >0.4, and >0.5 were selected for analysis (Table V). The threshold of >0.3 proved most sensitive, identifying 96% to 100% of fetuses with postnatally confirmed congenital hydronephrosis.

Table V. Transverse diameter of pelvis/kidney versus risk of postnatal congenital hydronephrosis

Transverse diameter, pelvis/kidney ratio	*14-23 wk*		*24-32 wk*		*33-42 wk*	
	Sensitivity (%)	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*
>0.30	96	51	100	37	100	26
>0.40	72	51	86	35	88	27
>0.50	52	41	60	31	77	23

Table VI. Kidney circumference/abdominal circumference versus risk of postnatal congenital hydronephrosis

Kidney circumference/abdominal circumference ratio	*14-23 wk*		*24-32 wk*		*33-42 wk*	
	Sensitivity (%)	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*	*Sensitivity (%)*	*False-positive rate (%)*
>0.28	95	49	89	33	59	26
>0.30	90	44	76	32	48	22
>0.32	76	43	58	27	41	0

Kidney/abdominal circumference ratio. Thresholds of >0.28, >0.30, and >0.32 were examined (Table VI). A threshold of >0.28 appeared optimal although sensitivity decreased to only 59% in late pregnancy.

Renal parenchymal thickness. Although ultrasonographically measured parenchymal thickness might potentially reflect cortical atrophy, no threshold that accurately predicted infant outcome could be identified.

Calicectasis. Dilated calyces were noted prenatally in only 47% (28/60) of hydronephrotic kidneys. While not highly sensitive for prenatal diagnosis of postnatally confirmed congenital hydronephrosis, calicectasis did correlate with morbidity. As noted in Table VII, surgery and renal compromise were significantly more common in fetuses with dilated calyces than in fetuses without calicectasis.

Table VII. Impact of calicectasis on postnatal outcome

	Normal calyces	*Dilated calyces*
Kidneys (No.)	32	28
Surgery required (%)	41	79
Renal compromise (%)	28	50
Surgery with or without compromise (%)	56	89

Comment

Ultrasonographic recognition of a prominent fluid collection within the fetal renal pelvis represents a diagnostic dilemma. False-positive diagnosis of fetal hydronephrosis may lead to unwarranted parental anxiety, as well as unnecessary and expensive diagnostic studies after delivery. At the same time failure to recognize and promptly treat clinically significant obstructions may result in impaired renal function. Several ultrasonographic criteria have been proposed for prenatal diagnosis of congenital hydronephrosis; however, this is the first investigation to compare these parameters in the same population and correlate their accuracy in predicting infant outcome.

Anteroposterior renal pelvic diameter is perhaps the most commonly used technique for diagnosing congenital hydronephrosis. Both Arger et al.[4] and Grignon et al.[5] considered an anteroposterior diameter ≥10 mm to be predictive of abnormal outcome. In addition, Arger et al.[4] suggested follow-up scans in any fetus with an anteroposterior diameter of ≥5 mm. Data from the current investigation indicate that a threshold of 10 mm is too high. For example, 68% of fetuses with postnatally confirmed congenital hydronephrosis would be missed before 24 weeks' gestation (Table III). In contrast, a threshold of 4 mm yields a sensitivity of 100%. While the false-positive rate is higher with a 4 mm threshold, the goal is to identify all "at risk" fetuses. In addition, the false-positive rate declines as gestational age advances, falling to only 24% near term. After 33 weeks' gestation, a threshold of 7 mm can be used while still maintaining 100% sensitivity.

The second analyzed ultrasonographic parameter was the ratio of the anteroposterior diameters of the renal pelvis and kidney. Both Arger et al.[4] and Kleiner et al.[6] suggested that a ratio exceeding 0.50 should be used for prenatal diagnosis of congenital hydronephrosis. Before 24 weeks' gestation, this threshold would fail to detect 60% of postnatally confirmed lesions; after 24 weeks, nearly 50% of affected fetuses would remain undetected (Table IV). To achieve a sensitivity of 90% to 100%, an anteroposterior pelvis/kid-

Table VIII. Anteroposterior pelvic diameter: Thresholds for counseling

	14-23 wk		*24-32 wk*		*33-42 wk*	
Anteroposterior pelvic diameter (mm)	*CH* (%)	*S/C* (%)	*CH* (%)	*S/C* (%)	*CH* (%)	*S/C* (%)
<3	0	0	0	0	0	0
4-6	41	19	38	13	0	0
7-9	53	40	33	6	67	50
>10	82	73	86	72	82	59

CH, Congenital hydronephrosis confirmed postnatally; *S/C*, postnatal surgery and/or evidence of renal compromise.

ney ratio of 0.28 is recommended. While this ratio is associated with a high false-positive rate in early pregnancy, progressive decline in the rate of false-positive results is noted as gestation advances.

The third examined biometric approach to prenatal diagnosis of congenital hydronephrosis was the ratio of the transverse diameters of the renal pelvis versus the kidney. Jeanty and Romero[7] described this technique in 1984 and considered a ratio ≥0.5 to be abnormal. In our study population this threshold identified only 52% of confirmed lesions before 24 weeks' gestation and missed 23% to 40% of affected fetuses during later pregnancy (Table V). A transverse pelvis/kidney ratio of 0.30 appeared more ideal, identifying nearly 100% of cases at all gestational ages. As with previously cited techniques, the false-positive rate declines with advancing gestational age.

The kidney circumference/abdominal circumference ratio was originally described by Grannum et al.[8] Although this method was developed as a means of quantifying renal enlargement in cystic kidney disease, an increased ratio also might be anticipated in congenital hydronephrosis. On examination of our data, a kidney circumference/abdominal circumference ratio of 0.28 appeared optimal during the midtrimester although sensitivity decreased to only 59% during late pregnancy (Table VI).

Of all analyzed techniques for prenatal diagnosis of congenital hydronephrosis, the anteroposterior diameter of the renal pelvis is simplest and has sensitivity and false-positive rates that are equal or superior to those of other ultrasonographic approaches. Follow-up studies are warranted in 3 to 4 weeks if an anteroposterior pelvic diameter is ≥4 mm before 33 weeks or ≥7 mm after 33 weeks. Postnatal follow-up studies are recommended if persistent renal pelvic dilatation is evident. Neonatal ultrasonographic evaluation should be postponed for several days after delivery to assure that the infant is well hydrated and the full extent of any potential obstruction appreciated. If abnormal, additional diagnostic studies including intravenous pyelography and renal scintigraphy may be helpful in further defining the lesion, as well as in delineating appropriate medical management.

Table VIII provides useful information for counseling parents when a diagnosis of congenital hydronephrosis is suspected. For example, before 24 weeks' gestation, there is a 41% chance of postnatally confirmed congenital hydronephrosis if the anteroposterior pelvic diameter is between 4 and 6 mm and a 19% risk for impaired renal function and/or surgery. The risk for adverse outcome increases to 40% if the anteroposterior diameter is between 7 and 9 mm and approaches 73% if ≥10 mm.

It is clear from this study that congenital hydronephrosis is a clinically significant birth defect. Overall, 69% (31/45) of affected infants had related morbidity as defined by abnormal renal function and/or the need for surgical intervention. We hope this investigation clarifies appropriate ultrasonographic criteria for diagnosis of congenital hydronephrosis and provides useful guidelines for counseling and management of this common congenital malformation.

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Endomyocardial ultrastructural findings in preeclampsia

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Ultrastructural findings in endomyocardial biopsy specimens obtained during cardiac catheterization in a patient with severe preeclampsia are described. Intramyocardial vessels revealed prominent swelling of the endothelial cell cytoplasm. In addition, cardiac myocyte mitochondria showed swelling and clearing within the matrix. Endomyocardial ultrastructural injury occurs in severe preeclampsia. (AM J OBSTET GYNECOL 1991;165:389-91.)

Key words: Preeclampsia; ultrastructure; injury, vascular

Although the exact cause of preeclampsia remains unknown, there are specific ultrastructural aspects that are consistently detected when tissues from patients with preeclampsia are studied. Presented are ultrastructural findings of the left ventricular endomyocardium from a patient with severe preeclampsia.

Case report

A 31-year-old white woman, gravida 4, para 1, aborta 2, presented at 35 weeks 5 days' gestation with a 4-day history of worsening shortness of breath. Pulmonary edema was diagnosed and intravenous furosemide and supplemental oxygen were initiated without improvement. Nasotracheal intubation was performed and mechanical ventilation was begun. A femoral arterial line was inserted for blood pressure and arterial blood gas monitoring. A nitroprusside drip was initiated at a dose

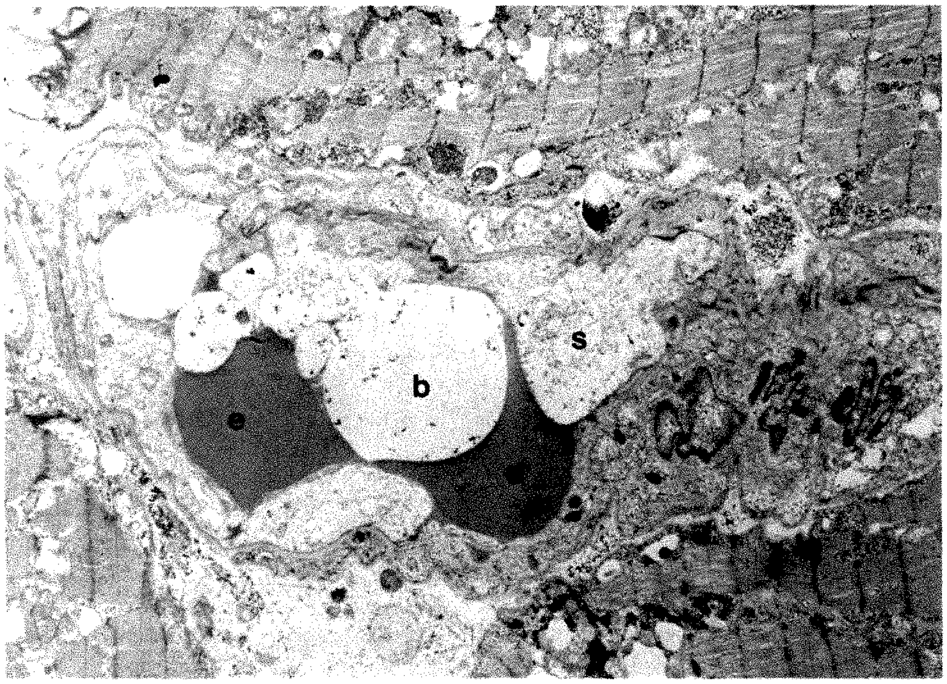

Fig. 1. Electron micrograph of intramyocardial vessel showing endothelial cell swelling *(s)*, blebs of disrupted endothelial cell cytoplasm *(b)*, and erythrocytes *(e)*. Glutaraldehyde fixation and Epon embedment. (Uranyl acetate-lead citrate. Original magnification ×6000, reduced 24%.)

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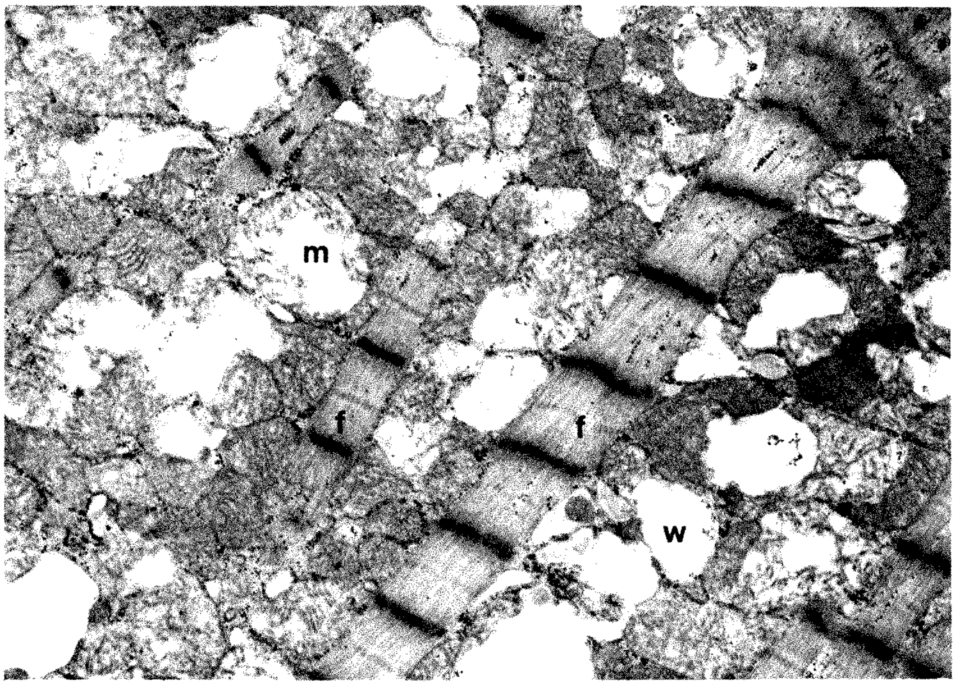

Fig. 2. Electron micrograph of cardiac myocyte cytoplasm showing extensive acute change in mitochondria, primarily including swelling and clearing of matrix *(m)* and laminated roll or whorls *(w)*. Myocardial fiber *(f)*. Glutaraldehyde fixation and Epon embedment. (Uranyl acetate–lead citrate. Original magnification × 17,000, reduced 24%.)

of 0.25 μg/kg/min to reduce afterload. Systolic blood pressure rapidly fell to 50 mm Hg. Blood pressure failed to recover despite discontinuation of the nitroprusside and utilization of dopamine and dobutamine infusions. The peripheral pulses were diminished. An intraaortic balloon counterpulsation device (9.5 F, Data Scope Corp., Paramus, N.J.) was inserted through the femoral artery. Initial setting was 1 : 1.

Invasive hemodynamic monitoring findings were consistent with pulmonary edema. An echocardiogram revealed normal chamber dimension; however, there was impaired global systolic function with an ejection fraction of 35%. Intravenous furosemide was continued, resulting in a brisk diuresis. Within 12 hours of admission, the clinical "picture" was more consistent with severe preeclampsia, with a mean arterial blood pressure of 127 mm Hg, proteinuria (3 +) and a serum uric acid level of 9.4 mg/dl. Intravenous magnesium sulfate was administered and a repeat cesarean section was performed. A 2640 gm infant was delivered with Apgar scores of 8 and 9 at 1 and 5 minutes, respectively.

On postoperative day 1, the patient underwent extubation. Mean arterial pressure decreased to 93 mm Hg. Cardiac output ranged from 4.7 to 6.2 L/min. Afterload reduction was accomplished with hydralazine and captopril. Intraaortic balloon counterpulsation was also discontinued on the first postoperative day.

On postoperative day 7, because of the atypical presentation and to evaluate for peripartum cardiomyopathy, the patient underwent a cardiac catheterization and left ventricular endomyocardial biopsies. Findings included left ventricular dysfunction with anterolateral hypokinesis, but with preserved left ventricular systolic and diastolic function. No valvular disease was identified, and minimal coronary artery disease was present. Microscopic examination of the endomyocardial biopsy specimen revealed myocardial fiber hypertrophy with focal interstitial fibrosis. There was neither myocarditis nor evidence of endocardial fibrosis, amyloidosis, or granulomas. Electron micrographs of the myocardium revealed a diffuse and extensive degenerative process. Small blood vessels revealed prominent swelling of endothelial cell cytoplasm with luminal narrowing, bleb formation, and trapping of red blood cells (Fig. 1). In addition, cardiac myocyte mitochondria showed swelling and clearing within the matrix, while the intramyocardial cristae retracted against the lateral membrane (Fig. 2).

Comment

Microvascular abnormalities in the heart of this patient were similar to changes seen in other organ systems of women with preeclampsia. Ultrastructural changes in the placental bed of patients with preeclampsia include swollen and eroded endothelium with reduction of the lumen.[1] Electron microscopy of endomyocardial biopsy specimens from our patient showed a similar swelling of endothelial cells with capillary luminal narrowing. The ultrastructural changes of endothelial injury from preeclampsia are postulated to be the result of both diffuse cytoplasmic and organellar swelling.[1] This swelling may then lead to erosion of the endothelium with progression to whole-cell desquamation.

The ultrastructural changes in mitochondria were similar to those recently described by Shanklin and Sibai.[2] Mitochondria were diffusely enlarged with clearing within the matrix and frequently displayed cristae bent against the lateral membrane. In the more advanced forms of disruption, a laminated roll or whorl was noted.

This case report demonstrates that the previously described ultrastructural changes in the endothelium of the placental bed, uterine boundary vessels, and renal glomeruli from patients with preeclampsia also may be noted in the microvasculature of the heart on endomyocardial biopsy. In addition, recently described changes in myometrial smooth muscle cell mitochondrial ultrastructure observed in patients with preeclampsia also were noted in heart muscle cells. Further study is indicated to determine whether comparable vascular injury occurs in other body sites during preeclampsia, as well as to investigate the organelle changes responsible for these findings.

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Additional references are available from the authors on request.

High risk of human papillomavirus infection and cervical squamous intraepithelial lesions among women with symptomatic human immunodeficiency virus infection

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We investigated the relationship of human papillomavirus (by cervicovaginal lavage and Southern blot), human immunodeficiency virus, and squamous intraepithelial lesions in 96 high-risk women in the Bronx, New York. Antibodies for human immunodeficiency virus were detected in 51 (53%) women. Of the 33 women with symptomatic human immunodeficiency virus infection, 23 (70%) had human papillomavirus infection compared with 4 of 18 (22%) asymptomatic women who were human immunodeficiency virus seropositive and 10 of 45 (22%) uninfected women ($p < 0.0001$). The rate of squamous intraepithelial lesions was 52% (14 of 27) for women with both viruses detected, 18% (6 of 34) for women with either virus detected, and 9% (3 of 35) for uninfected women. Among symptomatic human immunodeficiency virus–infected women, a strong association between human papillomavirus infection and squamous intraepithelial lesions was demonstrated (odds ratio, 12; 95% confidence interval, 1.3 to 108). Risk was highest for younger women from ethnic or racial minority groups. Advanced human immunodeficiency virus–related disease, with its associated immunosuppression, seems to exacerbate human papillomavirus–mediated cervical cytologic abnormalities. Public health measures are needed to provide Papanicolaou smear screening and appropriate clinical follow-up and treatment for women at high risk for human immunodeficiency virus infection. (AM J OBSTET GYNECOL 1991;165:392-400.)

Key words: Human immunodeficiency virus, human papillomavirus, cervix, sexually transmitted diseases, squamous intraepithelial lesions

Immunosuppression, whether from chemotherapy, malignancy, or infection, has been shown to be associated with higher than expected rates and advanced pathologic consequences of human papillomavirus (HPV) infection.[1-4] Immunosuppressed women from a wide range of age, racial, and socioeconomic groups have been reported to manifest greater HPV frequency and pathogenicity.[3,4] Therefore the cervix is likely to be a target organ for HPV-related pathologic conditions among women who have been immunosuppressed by a variety of causes. Studies in pregnant women, renal allograft recipients, Hodgkin's disease patients, and other women on a regimen of immunosuppressive drugs have suggested a dose-response relationship between severity of HPV-induced lesions and increased immunosuppression.[5,6] In addition, immunosuppressed women often respond poorly to conventional treatment for HPV-induced cytologic lesions, and residual infection often leads to the progression of a cytologic lesion even after treatment.[1,2]

Women with human immunodeficiency virus (HIV) infection have been reported to show a high prevalence of cervical intraepithelial neoplasia or squamous intraepithelial lesions, although most published or presented studies to date have not included a comparable uninfected control group.[6] A few controlled studies have suggested an association of HIV with cervical cytologic abnormality.[7-13] Cytologic evidence from these studies suggests that HIV infection may promote the pathologic consequences of HPV, which is analogous to the course of HPV infection among other immunosuppressed patients.[7,8] HIV may affect local cervical immunity by diminishing quantity or function of Langerhans' cells, a plausible mechanism for pathophysiologic changes.[6,14,15] Preliminary reports using

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molecular diagnosis of HPV in some studies suggest that advanced HIV-related clinical disease or immunosuppression seems to predispose to higher rates of cervical intraepithelial neoplasia,[6, 13, 16-18] including a preliminary report of a subset of the women reported here.[19] However, other preliminary studies found no such association.[20, 21] The strong relationship that exists between HPV, HIV, and anal dysplasia among homosexual men who engage in unprotected receptive anal intercourse[22, 23] suggests a common HIV immunosuppression–mediated mechanism of HPV-related neoplastic changes in selected genital epithelial tissues in both women and men.

This study was designed to investigate the hypothesis that HIV-infected women who are also infected with HPV, detected by molecular hybridization, are more likely to have cervical cytologic abnormalities than are women with only one or neither virus. Important potential confounding factors are considered. Additionally, we have sought to determine whether stage of HIV infection, i.e., asymptomatic or symptomatic, may be associated with frequency and severity of cervical cytologic abnormalities.

Methods

Subjects. The subjects included 97 women with known HIV serologic status, 96 of whom had cervicovaginal lavage samples that were sufficient for HPV determination and were thus included in this analysis. The protocol was approved by the hospital institutional review board for the protection of human subjects. Seventy-three were women with a history of heroin use attending a Bronx, New York, methadone maintenance program affiliated with a major medical center and enrolled in a longitudinal study of the natural history of HIV infection.[19] Twenty-two participants were recruited from women who were enrolled in a study of HIV transmission among heterosexual partners of intravenous drug users at the same medical center.[7] One woman was a transfusion recipient of HIV-seropositive blood, who was included because she was from the same geographic and socioeconomic background as the other subjects. HIV antibody status was determined by enzyme immunoassay (ELISA, DuPont) and confirmed by Western blot; positive results by both methods were noted in 51 (53%) of the 96 subjects.

Consecutive subjects were enrolled on the basis of availability of study personnel and provision of informed consent at the time of their visits. Women in the methadone program who were coming for annual physical examination and women who were enrolled in a heterosexual transmission study were recruited at the time of a research visit. A few high-risk women were recruited from the infectious disease clinic or medical ward of the hospital at a time when a Papanicolaou smear was medically indicated. All sexually active women with known or obtainable HIV serologic results from these recruitment environments were deemed eligible for the study.

After written informed consent was obtained, a brief questionnaire was administered to obtain demographic characteristics and smoking and gynecologic history. A pelvic examination was performed and a Papanicolaou smear was obtained with a cotton swab and a wooden Ayres spatula. Endocervical samples were then collected for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and herpes simplex virus cultures with a calcium alginate swab on an aluminum shaft for chlamydia and a cotton swab for gonorrhea and herpes simplex virus. Exfoliated cervicovaginal cells were obtained for HPV determination by cervicovaginal lavage as previously described.[19, 24-26]

Patient health status (e.g., rashes, chancres) and syphilis serologic results by Venereal Disease Research Laboratory test or reagin precipitin reaction were assessed at or near the time of the pelvic examination. If a positive test was noted, a fluorescent treponemal antibody test was performed. Syphilis was diagnosed if primary or secondary lesions were present with positive results for all tests or if the Venereal Disease Research Laboratory test or reagin precipitin reaction titer had not fallen after prior treatment for clinically confirmed syphilis.

Cytology. Papanicolaou smears were interpreted and classified, by personnel who were blinded to patient HIV and HPV status, on the basis of the Bethesda Terminology and Classification system for cervical and vaginal cytology.[27] All abnormal and most normal smears were confirmed by one of the authors (K.S.). For analyses, we compared subjects with smears considered benign (negative or showing only reactive or reparative changes) with those having "positive" smears (smears providing evidence of squamous intraepithelial lesions or cancer). To provide a more striking clinical comparison, most analyses were repeated to exclude subjects from the analysis whose Papanicolaou smears showed only reactive or reparative changes; these repeat analyses contrasted characteristics of women with completely negative smears with those of women with squamous intraepithelial lesions. Women were referred for colposcopic examination where clinically indicated; however, follow-up is incomplete and results of additional gynecologic evaluations are therefore not presented.

Molecular biology. Deoxyribonucleic acid (DNA) extracted from cervicovaginal cells was tested for human papillomavirus DNA by *Pst* I restriction analysis and Southern blot hybridization, with a mixed probe of HPV DNA types 11, 16, and 18. Hybridizations were performed under conditions of low stringency (i.e.,

Table I. Sociodemographic and behavioral characteristics and presence of concurrent sexually transmitted diseases, by HIV and HPV infection status, $N = 96$

	HIV positive		*HIV negative*	
	HPV positive (n = 27)	*HPV negative (n = 24)*	*HPV positive (n = 10)*	*HPV negative (n = 35)*
Mean age (yr, mean ± SD and range)	33.9 ± 4.8 (24-42)	34.1 ± 5.8 (24-48)	34.7 ± 3.9 (29-40)	35.5 ± 7.2 (23-58)
Race (No. and column %)				
White	2 (7.4)	4 (17)	6 (60)	13 (37)
Black	8 (30)	5 (21)	2 (20)	4 (11)
Hispanic	17 (63)	15 (63)	2 (20)	18 (51)
Education (yr, mean ± SD and range)	11.3 ± 2.5 (7-17)	10.8 ± 2.6 (5-16)	11.6 ± 1.8 (10-16)	11.5 ± 2.6 (5-17)
Patient source (No. and column %)				
Methadone clinic	17 (63)	14 (58)	10 (100)	27 (77)
Other	10 (37)	10 (42)	0 (0)	8 (23)
Smoking history (pack yr*)				
Median	18.1	9.3	15.0	18.9
Geometric mean	18.5	10.9	14.1	15.4
Women pregnant at time of examination (No. and %)	3 (11)	1 (4.2)	2 (20)	5 (14)
Mean age at first coitus (yr, mean ± SD and range)	15.8 ± 2.3 (10-22)	15.6 ± 2.7 (11-25)	16.4 ± 1.4 (14-19)	16.2 ± 3.2 (9-27)
Median No. of sexual partners				
Lifetime	7.0	5.0	5.0	6.0
Last year	1.0	1.0	1.0	1.0
Concurrent sexually transmitted diseases (No. and %)†				
Chlamydia	1/27 (3.7)	1/24 (4.2)	0/10 (0)	2/35 (5.7)
Gonorrhea	1/23 (4.3)	1/23 (4.3)	0/10 (0)	1/35 (2.9)
Syphilis	5/22 (23)	4/23 (17)	2/10 (20)	1/35 (2.9)
Herpes simplex virus	0/27 (0)	0/24 (0)	0/10 (0)	0/35 (0)
Oral contraceptive use (yr, mean ± SD and range)	1.4 ± 3.8 (0-17)	2.6 ± 3.9 (0-14)	3.0 ± 3.6 (0-10)	1.7 ± 3.1 (0-16)
Pregnancies (No., mean ± SD and range)	3.9 ± 2.2 (1-8)	5.3 ± 1.8 (2-9)	4.1 ± 2.2 (0-7)	4.2 ± 2.4 (0-8)

*Estimated number of packs of cigarettes per day times number of years smoked.

†Some data missing because of inadequate or lost specimens.

tm = −40° C), followed by a low-stringent wash, after which filters were exposed for 1 to 3 days. Then a subsequent wash at high stringency was followed by exposure for 1 to 2 weeks.[24] All HPV DNA determinations were made without the knowledge of any laboratory or clinical findings.

Clinical assessment. Chart review of all medical records of the 51 HIV-infected women was performed to assess signs and symptoms of HIV disease, and classification was made according to the Centers for Disease Control scheme.[28] On the basis of data from the clinical and research records, women were classified into one of four groups: group 1, acute infection; group 2, asymptomatic infection; group 3, persistent generalized lymphadenopathy; and group 4, other diseases associated with HIV infection. No patient had evidence of acute infection, and patients in groups 3 and 4 were combined into one category for purposes of analysis, comparing HIV-infected persons without signs or symptoms (termed *asymptomatic*) with HIV-infected persons with signs or symptoms (termed *symptomatic*).

Seven subjects (all symptomatic HIV infected) were being treated with zidovudine and three others were taking acyclovir as prophylaxis for recurrent HSV infection. Eight subjects were participating in a double-blind, placebo-controlled trial of zidovudine efficacy in asymptomatic, HIV-seropositive persons. These 18 HIV-infected women had behavioral and sociodemographic characteristics similar to those of the other 33 HIV-positive patients and were analyzed with their appropriate clinical symptom group.

Biostatistics. Standard statistical methods were used for data analysis including χ^2 tests (with Yates' correction for 2 × 2 tables) or two-tailed Fisher's exact tests when sample size did not permit use of the χ^2. Odds ratios and Mantel-Haenszel odds ratios with 95% confidence intervals were used to measure strength of stratum-specific and overall associations in multivariate analyses. One-way analysis of variance was used to compare average age of subgroups.

Evidence of squamous intraepithelial lesions at cervical cytologic testing was the outcome studied. Women

Table II. Association of symptomatic and asymptomatic HIV and HPV among 96 high-risk women

	HIV							
	Seropositive, symptomatic		*Seropositive, asymptomatic*		*Seronegative*		*Total*	
	No.	*Column %*	*No.*	*Column %*	*No.*	*Column %*	*No.*	*Column %*
HPV								
Positive	23	70	4	22	10	22	37	39
Negative	10	30	14	78	35	78	59	61
TOTAL	33	100	18	100	45	100	96	100

$p < 0.0001$, by χ^2.

were grouped into six categories on the basis of the two major independent variables: (1) symptomatic HIV-infected and HPV-infected, (2) symptomatic HIV-infected and HPV-uninfected, (3) asymptomatic HIV-infected and HPV-infected, (4) asymptomatic HIV-infected and HPV-uninfected, (5) HIV-uninfected and HPV-infected, and (6) HIV-uninfected and HPV-uninfected. For some analyses, categories 1 and 3 (HIV-infected and HPV-infected), 2 and 4 (HIV-infected and HPV-uninfected), and 5 and 6 (HIV-uninfected) were collapsed. One summary analysis combined categories 3 through 6 into one group (asymptomatic HIV-infected or HIV-uninfected). Given the heterogeneous rates for HIV, HPV, and squamous intraepithelial lesions in various racial and ethnic categories (see Results), the key analyses that associate HIV and HPV and that associate HIV, HPV, and Papanicolaou smear results were stratified by race. Age was controlled with stratum-specific analyses comparing women <34 years old (median, See results) with women ≥34 years old.

Results

Pregnancy status, sexual behavior, and sociodemographic characteristics including age and education were similar between groups of subjects (Table I). Nonwhite subjects were disproportionately represented among HIV-infected women, as has been reported previously.[29]

HIV and HPV status. The clinical status of HIV infection among the 51 HIV-seropositive women included 18 asymptomatic subjects and 33 symptomatic subjects with persistent generalized lymphadenopathy, oral candidiasis, or acquired immunodeficiency syndrome. Thirty-seven (39%) of the study participants had cervicovaginal lavage specimens that were positive for HPV infection. Among the 51 HIV-infected women, 27 (53%) of the cervicovaginal samples contained HPV DNA, whereas 10 (22%) of 45 HIV-seronegative women were infected with HPV ($p < 0.005$). This association was attributed in full to the excess in HPV infection among symptomatic women (70%); the frequency of HPV infection in asymptomatic HIV-infected women (22%) was identical to that among HIV-uninfected women (Table II).

Cervical cytology. Seventeen of 51 (33%) HIV-seropositive women had Papanicolaou smear abnormalities demonstrating squamous intraepithelial lesions, compared with six of 45 (13%) HIV-seronegative women ($p < 0.05$). Of HPV-infected women, 17 (46%) of 37 had squamous intraepithelial lesions, whereas 6 (10%) of 59 HPV-uninfected women had squamous intraepithelial lesions ($p < 0.0005$).

Among symptomatic HIV-infected women, a significant association between HPV infection and cervical lesions showing squamous intraepithelial lesions was noted (odds ratio, 12; 95% confidence interval, 1.3 to 108; Table III). With smaller numbers of patients and fewer adverse outcomes, the associations of HPV infection and squamous intraepithelial lesions for asymptomatic HIV-infected and HIV-uninfected women showed a positive trend but did not reach statistical significance (Table III). The overall association of HPV and positive Papanicolaou smear was significant, controlling for HIV infection and clinical status (Mantel-Haenszel odds ratio, 5.8; 95% confidence interval, 1.5 to 18).

The rate of cervical abnormality was 57% (13 of 23) for women infected with both HIV and HPV and symptomatic for their HIV infection, 18% (7 of 38) for women infected with either virus alone or infected with both viruses but asymptomatic for HIV infection, and 8.6% (3 of 35) for women not infected with either virus. In a comparison of women infected with both HIV and HPV and symptomatic for HIV infection with women not infected with either virus, a relative odds of 13.9 (95% confidence interval, 3.3 to 59) for squamous intraepithelial lesions was noted.

When women with Papanicolaou results typical of reactive or reparative changes only are excluded, the associations of HPV and abnormal Papanicolaou smear were still significant in the women with symptomatic HIV infection (odds ratio, 13; 95% confidence interval, 1.3 to 133). No significant associations of HPV and squamous intraepithelial lesions were noted in the asymptomatic HIV-infected or the HIV-uninfected

Table III. Association of squamous intraepithelial neoplasia with presence of HPV DNA in women with and without HIV infection and symptoms, $N = 96$

	Papanicolaou smear		*Total*	
	Squamous intraepithelial neoplasia	*No squamous intraepithelial neoplasia*	*No.*	*Column %*
*Symptomatic HIV-seropositive women**				
HPV				
Positive	13	10	23	70
Negative	1	9	10	30
TOTAL (No. and row %)	14 (42)	19 (58)	33	100
Asymptomatic HIV-seropositive women†				
HPV				
Positive	1	3	4	22
Negative	2	12	14	78
TOTAL (No. and row %)	3 (17)	15 (83)	18	100
HIV-seronegative women‡				
HPV				
Positive	3	7	10	22
Negative	3	32	35	78
TOTAL (No. and row %)	6 (13)	39 (87)	45	100

Mantel-Haenszel odds ratio, 5.8; 95% confidence interval, 1.5, 18.
*Odds ratio, 12; 95% confidence interval, 1.3 to 108.
†Odds ratio, 2.0; 95% confidence interval, 0.1 to 30.
‡Odds ratio, 4.6; 95% confidence interval, 0.8 to 28.

women, although the overall Mantel-Haenszel odds ratio was significant (5.4; 95% confidence interval, 1.4 to 18). The rates of cervical abnormality were 76% (13 of 19) for women infected with both HIV and HPV and symptomatic for HIV infection, 21% (7 of 33) for women infected with either virus or infected with both viruses but asymptomatic for HIV infection, and 11% (3 of 27) for women not infected with either virus. Women infected with both HIV and HPV and symptomatic for HIV infection were 17 times more likely to have squamous intraepithelial lesions on Papanicolaou smear than women not infected with either virus (odds ratio, 17; 95% confidence interval, 3.7 to 81).

Four women had high-grade squamous intraepithelial lesions (equivalent of grade 2 or 3 cervical intraepithelial neoplasia). All four were infected with HIV. Three of the women were infected with HPV and had symptomatic HIV infection. The fourth women was asymptomatic for HIV and did not have HPV detected.

Age and race. Sizes of individual analytic cells were small with further subgroup analyses. Significant differences in age were not noted in our population of young and middle-aged women in various comparisons: mean age in years by Papanicolaou group ($p = 0.3$), by HIV status ($p = 0.3$), by HPV status ($p = 0.5$), and by combined HIV and HPV status ($p = 0.8$).

Among symptomatic women with HIV infection, an association between abnormal cervical status and HPV was most evident among black and Hispanic women <34 years old (odds ratio, 11; 95% confidence interval, 0.8 to 148). Nonwhite and white women ≥34 years old with symptomatic HIV infection did not have a significant association of HPV with squamous intraepithelial lesions, although numbers were small (odds ratio, 4.3; 95% confidence interval, 0.2 to 108; Table IV). No white women <34 years old had symptomatic HIV infection. An overall association of HPV infection with squamous intraepithelial lesions was noted among symptomatic HIV-infected women, stratified by this combination of age and race or ethnicity (Mantel-Haenszel odds ratio, 7.2; 95% confidence interval, 1.01 to 57). This association was not explained by other measured factors, including age at first intercourse, other sexually transmitted diseases, and number of sexual partners (Table I).

Similarly, the association of HPV and HIV infections was strongest among younger black and Hispanic women (Table V). Black and Hispanic women <34 years old had a significant association of HIV and HPV infections (odds ratio, 18; 95% confidence interval, 2.0 to 161) compared with all other women (odds ratio, 2.2; 95% confidence interval, 0.7 to 6.5). A trend for highest rates of squamous intraepithelial lesions and strongest HIV and HPV correlations among Hispanic subjects was noted.

Other sexually transmitted infections. The presence of a concurrent positive genital culture for *Chlamydia* ($n = 4$) or gonorrhea ($n = 3$) was not associated with increased risk of HIV or HPV infection or with cervical

Table IV. Association of cervical cytologic abnormality with presence of HPV DNA in women with symptomatic HIV infection, by race and age, $N = 33$

	Papanicolaou smear	*Squamous intraepithelial lesions*	*No squamous intraepithelial lesions*	*Total*	
				No.	*Column %*
Black and Hispanic, <34 yr old*†					
HPV positive, HIV symptomatic		11	3	14	78
HPV negative, HIV symptomatic		1	3	4	22
Black and Hispanic, ≥34 yr old					
HPV positive, HIV symptomatic		1	6	7	64
HPV negative, HIV symptomatic		0	4	4	36
White and other, ≥34 yr old					
HPV positive, HIV symptomatic		1	1	2	50
HPV negative, HIV symptomatic		0	2	2	50
All races, ≥34 yr old‡					
HPV positive, HIV symptomatic		2	7	9	60
HPV negative, HIV symptomatic		0	6	6	40

Mantel-Haenszel odds ratio, 7.2; 95% confidence interval, 1.01 to 57. Calculated with strata black and Hispanic and all races; 0.5 was added to each cell of all races.

*No white women who were <34 years old had symptomatic HIV infection.

†Odds ratio, 11; 95% confidence interval, 0.8 to 148.

‡Odds ratio, 4.3; 95% confidence interval, 0.2 to 108 (0.5 added to each cell).

Table V. Association of HPV DNA with HIV infection, by race and age, $N = 96$

	HIV positive	*HIV negative*	*Total*	
			No.	*Column %*
Black and Hispanic, <34 yr old*				
HPV positive	15	1	16	42
HPV negative	10	12	22	58
White and other, <34 yr old				
HPV positive	1	5	6	50
HPV negative	1	5	6	50
Black and Hispanic, ≥34 yr old				
HPV positive	9	3	12	38
HPV negative	10	10	20	63
White and other, ≥34 yr old				
HPV positive	2	1	3	21
HPV negative	3	8	11	79
All white and other; black and Hispanic ≥34 yr old†				
HPV positive	12	9	21	36
HPV negative	14	23	37	64

Mantel-Haenszel odds ratio = 3.9, 95% confidence interval, 1.3 to 8.8; calculated with strata from all groups.

*Odds ratio, 18; 95% confidence interval, 2.0 to 161.

†Odds ratio, 2.2; 95% confidence interval, 0.7 to 8.8.

cytologic abnormality. None of the patients had a positive culture for herpes simplex virus. Nine patients with current evidence of syphilis were HIV seropositive, while three were HIV seronegative, representing a statistically insignificant trend toward an excess syphilis rate among HIV-infected women (Table I).

Comment

HIV, HPV, and squamous intraepithelial lesions. This study demonstrates that symptomatic HIV-infected women with HPV infection were at much higher risk for squamous intraepithelial lesions than any other subgroup.[6] This finding suggests that the immunosuppression associated with pathogenic consequences of HIV infection exacerbates HPV-mediated cervical pathogenesis.[16] Women with either HIV or HPV infection alone were at higher risk for squamous intraepithelial lesions than women without any infection, but women with both infections had an estimated relative odds of having squamous intraepithelial lesions nearly 12 times that of women with neither viral infection. The mechanism through which immunosuppression may activate and accelerate the pathogenic course of HPV infection is unclear. It is possible that immunodeficiency allows establishment of a chronic, active HPV infection, favoring the likelihood of oncogenic progression,[6, 23] or immunodeficiency may allow HPV to replicate to higher levels with more clinically aggressive lesions than in immunocompetent women, as has been noted in pregnant women.[5, 30] The ability of a local infection to persist and progress may rest on its ability to create a nurturing environment for itself by effecting depletion of Langerhans' cells and T lymphocytes in the epithelial tissue; conceivably, HPV or HIV infection could have a direct effect on Langerhans' cells, which are migrating cells that are responsible for picking up antigen and delivering it to the lymph nodes.[5, 14, 15] Additional areas for investigation include the effect of HPV type on disease progression in varying states of immune competence and the interaction of systemic and local immunologic responses in the modulation of HPV pathogenesis.

The analytic strategy in this work was designed to address three related issues: (1) to examine the association of HIV and HPV infection; (2) to see if the relationship between HPV and squamous intraepithelial lesions was modulated by either symptomatic or asymptomatic HIV infection, indicating a likely effect of immunosuppression; and (3) to see if race, age, other sexually transmitted diseases, or behavioral variables could account for, or otherwise affect, any of these virologic-clinicopathologic associations. An association of HIV and HPV was confirmed in the complete sample, but an increased rate of HPV infection was found only in the patients who were symptomatic for HIV infections (Table II). In our study, women with asymptomatic HIV infection were no more likely to be infected with HPV than were women without serologic evidence of HIV infection, although our statistical power was limited for asymptomatic seropositive women, who were comparatively underrepresented in the study sample. The principal research finding was the high risk for squamous intraepithelial lesions in the symptomatic HIV-infected women who were also infected with HPV. Finally, younger age and minority ethnic status were associated with increased risk for HIV, HPV, and squamous intraepithelial lesions. The biologic and behavioral origins of this latter observation are not known.

Since no excess risk for squamous intraepithelial lesions was noted for asymptomatic HIV-seropositive women, a threshold effect may be occurring in that only more profoundly immunosuppressed women experience increased cervical morbidity, presumably because of decreases in immunologic surveillance.[23] Alternatively, a dose-response effect is plausible with greatest risk associated with HIV and profound immunosuppression, less risk with HIV infection and less immunosuppression, compared with the baseline risk noted in a comparable population of women without HIV infection and presumably with a relatively normal immune status. Further study will be necessary to distinguish between these possibilities.

HPV-associated cervical disease should be added to the list of preventable opportunistic complications of importance in HIV-infected women. We should anticipate the possibility of an HIV-related cervical cancer epidemic among women in industrialized countries where cervical cancer rates are currently relatively low. This scenario is especially plausible if the course of HIV infection is prolonged because of antiretroviral therapy and prophylaxis for opportunistic infections, since these interventions might, in turn, permit manifestations of advanced cervical disease to occur in the setting of prolonged immunosuppression.[31, 32] Although good evidence exists for the role of both immunosuppression and HPV in cervical disease, we do not yet have the population-based data needed to fully assess the public health impact of these findings, e.g., future cervical morbidity among HIV-infected women and whether prognostic importance of the severe or intractable squamous intraepithelial lesions may be comparable to that of other opportunistic infections currently included in the acquired immunodeficiency syndrome surveillance definition. One issue immediately suggested by available data is the need for improved cervical surveillance of HIV-infected women, particularly those with HIV-related symptoms, with the Papanicolaou smear.

In developing countries with much higher baseline rates of cervical cancer, much higher frequency of other causes of immunosuppression and immune activation (malnutrition and coinfections, for example), and high prevalence of HIV in women, the concern is even greater. Papanicolaou smear screening is largely unavailable or unaffordable for poorer women in developing nations. While the findings in the present study need to be confirmed in developing countries,[12, 20] it is likely that results from such studies will ultimately reinforce the already obvious need for Papanicolaou smear screening and cervical treatment opportunities for women in developing nations as well as in many underserved communities in industrialized countries.[33]

HPV as a possible risk factor for HIV infection. Further study, of major public health importance, that should be pursued is whether HPV infection in cervical cells could facilitate HIV transmission through disruption of normal mucosal integrity or through altered local immune surveillance. Studies in small numbers of women have found evidence of decreased response of cervical lymphocytes to mitogens, decreased helper-to-suppressor T-cell ratios, and depletion of Langerhans' cells in cervical squamous epithelium infected by HPV.[5] HIV was first isolated from the cervix in 1986, and cell-associated infection is probable.[14, 15] Hence the role of

HPV in enhancing HIV infection, through the equivalent of microulcerations or areas of diminished local immune competence, should be studied prospectively in women at risk for HIV infection in the context of the many other cofactors that may facilitate HIV transmission. HPV is so common that even a modest relative risk for facilitation of HIV infection could translate into a large population-attributable risk, potentially making it an important cofactor in transmission, as has been reported for herpes simplex virus, chancroid, and syphilis, which cause larger, usually visible, but more uncommon genital ulcers.[5, 34]

Clinical policy implications. This study suggests that a number of public health policy decisions should be advocated:

1. Yearly Papanicolaou smear and gynecologic examination for women at high risk for HIV should be offered[35]; if symptomatic HIV infection or CD4+ cell counts $<200/mm^3$ occur, screen every 6 months.
2. Consider HPV-mediated cervical disease as an HIV-related opportunistic complication.
3. Offer ablative therapy for patients with high grade squamous intraepithelial lesions to avoid rapid progression of cervical disease.
4. Use state-of-the-art clinical and molecular methods to prospectively monitor high-risk women.
5. Expand Papanicolaou smear programs and appropriate therapies into the poorest communities, especially those with high HIV prevalence rates.
6. Study HPV as a potential cofactor for HIV transmission.
7. Replicate and expand the present findings, particularly in developing nations and with specific data on T-cell subsets.
8. Initiate prospective studies with improved clinical data using colposcopy.

The demographic evolution of the HIV epidemic suggests an increasing proportional representation of women.[30, 36, 37] The association of HIV, HPV, and squamous intraepithelial lesions and the continuing clinical advances in management of HIV disease all suggest that cervical disease and HPV infections are likely to become more prominent features in the clinical profile of women with HIV infection. Substantial costs in human and economic terms may be anticipated from cervical disease, particularly among women whose life spans are lengthened by treatments that do not reverse immunosuppression.

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Case report of laparoscopic cholecystectomy in the third trimester of pregnancy

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Laparoscopic cholecystectomy has not been reported during pregnancy. This is the first reported case of a cholecystectomy by laparoscopic technique in the third trimester of pregnancy. The patient (and her baby) had a rapid, uneventful recovery identical to that of a nonpregnant patient. (AM J OBSTET GYNECOL 1991;165:401-2.)

Key words: Laparoscopic cholecystectomy, third-trimester pregnancy

Gallstones that become symptomatic during pregnancy are usually managed conservatively, and when conservative management fails, an open cholecystectomy has been necessary. An upper midline incision is especially uncomfortable for the patient during the final weeks of pregnancy. The incidence of ventral hernias predictably increases, and the painful incision necessitates postoperative narcotics with resultant maternal pulmonary depression and fetal depression. Laparoscopic cholecystectomy avoids the incision with its resultant pain and complications and provides a great increase in the benefit/risk ratio for the patient.

Case report

A 27-year-old woman who was 31 weeks pregnant began to have right upper-quadrant pain, with nausea and vomiting, which was intermittent and was precipitated by eating. Ultrasonography of the gallbladder revealed multiple small stones with normal intrahepatic and common bile ducts. The gallbladder wall was of normal thickness.

Liver function tests and white blood cell count were normal. She was afebrile. Attempts at conservative management were unsuccessful, and the patient was unable to tolerate any diet except water for 4 days.

Surgical procedure. When the patient was under

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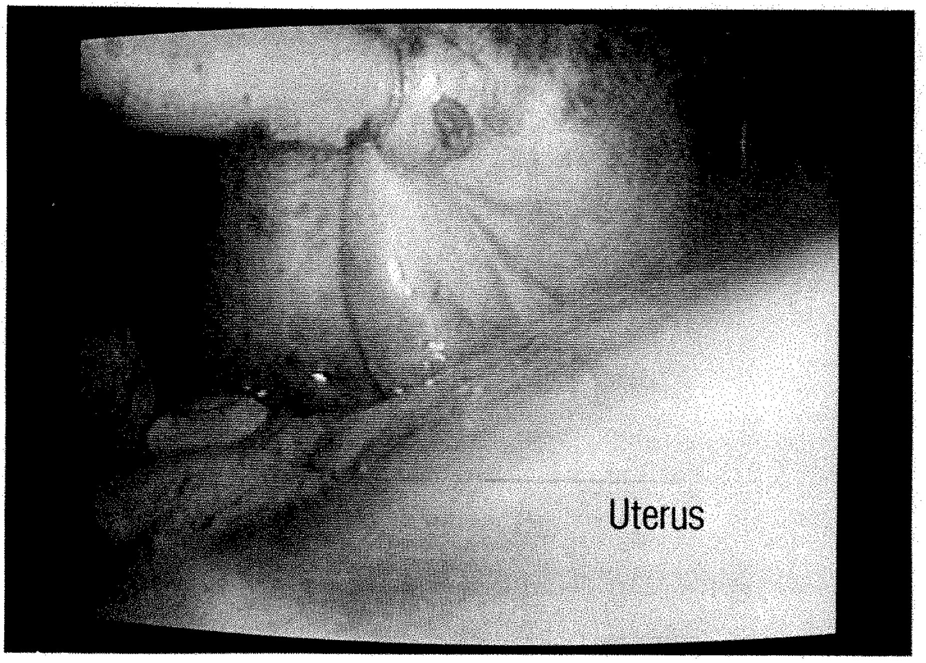

Fig. 1. Intraoperative view of gallbladder with 31-week gravid uterus in foreground.

general anesthesia, a 10 mm cannula was inserted just inferior to the umbilicus via the open (Hasson) method of laparoscopy. The patient was placed in reverse Trendelenburg position and was tilted to the left to facilitate exposure of the cystic duct and artery. A pneumoperitoneum was created with carbon dioxide to 14 mm Hg pressure. A 10 mm port with a zero-degree laparoscope was used with a camera and video monitor system. Another 10 mm port and two 5 mm ports were placed under direct vision. The gallbladder was dissected and removed by the standard, previously described method[1] (Fig. 1). The four puncture wounds were closed with subcuticular Dexon sutures.

The pathology report revealed chronic inflammation and more than 200 1 mm stones.

The patient was able to tolerate her usual diet the evening of the operation. She required only oral medication for pain and was discharged 48 hours after operation. There were no signs of fetal distress on monitoring during or after operation. A normal male infant, who weighed 6 pounds 4 ounces and who had Apgar scores of 8 and 9, was delivered by an uncomplicated, repeat low-segment cesarean section 10 weeks after the procedure.

Comment

Indications for and experience in laparoscopic cholecystectomy are rapidly increasing. Because the large abdominal incision is avoided with the laparoscopic procedure, the patient's postoperative course is markedly improved. Pain is controlled by oral medications, patients are able to eat a normal diet the same day, and most patients go home within 24 hours and are back at work within 1 week. In pregnant patients laparoscopic cholecystectomy has additional advantages. Fetal depression from intramuscular narcotics is eliminated, because postoperative pain is minimal. The potential atelectasis from hypoventilation that results from the combination of intraabdominal mass effect (gravid uterus) and incisional pain is nil.

The major complications related to an abdominal incision (wound infection, wound disruption, and ventral hernia), that occur especially during the last 2 months of pregnancy become moot.

Because this patient was out of bed within 8 hours after operation, pulmonary embolism predictably became a very remote possibility.

In this case return to a full diet in 8 hours eliminated any nutritional stress to the baby. In our experience of >100 consecutive laparoscopic cholecystectomies, 96% of patients have been able to eat a normal diet the same day.

It has been stated[2] that pregnancy is an absolute contraindication for laparoscopic cholecystectomy. No reasons have been given. An open approach to inserting the laparoscope avoids inadvertent injury to the uterus or intrabdominal contents. The other ports can then be inserted under direct visual control.

The issue of adequate visualization of biliary structures is always the determining factor for safe completion of the procedure. At 31 weeks' gestation, in this case at least, there was no difficulty, and we would not expect this to be an exception.

Intraoperative serial monitoring of arterial blood gas values in our laparoscopic cholecystectomy patients has shown little effect on P_{CO_2} or arterial pH and no effect on P_{O_2} or bicarbonate. There was no noticeable intraoperative or postoperative effect of increased abdominal pressure that resulted from carbon dioxide insufflation to 14 mm Hg, but long-term results are not available.

We believe that pregnancy is not a contraindication to a laparoscopic cholecystectomy, and the procedure provides marked benefits for the patient who needs removal of the gallbladder during pregnancy.

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Psychosocial support for maternal stress during pregnancy: Effect on birth weight

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In a previous study we showed that moderate to severe stress during pregnancy was inversely related to infant birth weight. Using the same criteria for stress (according to the Social Readjustment Rating Scale of Holmes and Rahe), we studied 86 white mothers with singleton pregnancies and with no known medical or obstetric risk factors for reduced birth weight. After strict randomization, data were analyzed for 43 mothers who received psychosocial support between enrollment at ±20 weeks and delivery at ±38 weeks and for 43 control mothers who received standard care at the antenatal clinic. In the supported group seven infants weighed <3000 gm at birth versus 18 control infants ($p = 0.008$), and analysis revealed that this effect was more the result of improved intrauterine growth than of prolongation of pregnancy. These findings are of little clinical relevance as far as the neonate is concerned, but they do indicate that psychosocial support has a significant effect on birth weight. It is possible that previous studies that have looked only for an effect on low- or very-low-birth-weight rates might have missed this clinically measurable benefit of counseling. (AM J OBSTET GYNECOL 1991;165:403-7.)

Key words: Psychosocial support, pregnancy, birth weight

A relationship between major life events and low birth weight (<2500 gm) has been described by several authors.[1-3] This led us to a preliminary study in 1985,[4] in which a group of 118 mothers who were delivered prematurely at the Johannesburg Hospital were shown to have an excess of moderate to severe stressors as identified by the Social Readjustment Rating Scale of Holmes and Rahe.[5] Our initial study was followed by a larger retrospective study of 535 mothers delivered at the same hospitals; moderate to severe stress factors were identified in 48% of the mothers, and a significant, inverse relationship was observed between cumulative stress score and birth weight.[6] Having thus confirmed an interaction between stress and birth weight in our local population, we faced the question whether stressed mothers attending the antenatal clinic would benefit from psychosocial support and whether such support would influence birth weight or gestational age. While descriptive studies have demonstrated the interaction between psychosocial stress and birth weight,[1-3, 7] as indicated in a recent overview,[8] no prospective, controlled trial has specifically evaluated the effects of intervention in mothers enrolled on the basis of psychosocial stress.

Subjects and methods

Data from the previous study[6] were used to determine sample size for this randomized, controlled study of support for mothers suffering from moderate to severe stress during pregnancy. Analysis of data from the previous study[6] indicated that a stress effect approached significance when a weight cutoff of 3000 gm was used; 25% of the 280 babies born to nonstressed mothers weighed <3000 gm versus 35% of the 255 babies born to those with stress. On the basis of these figures, the prospective study was designed with a minimum sample of 86 subjects required to determine whether intervention could achieve a 50% reduction in the number of babies weighing <3000 gm.

Mothers of singleton pregnancies were eligible for enrollment between the eighteenth and twenty-fifth weeks of pregnancy if they were free of medical or obstetric problems known to be associated with prematurity or low birth weight (LBW). In keeping with the objective of eliminating as many known causes of LBW (<2500 gm) as possible, the study was confined to white mothers; this was because of the known association between lower birth weight and black race.[9] As in the preliminary study,[6] stress was defined as any factor scoring ≥39 on the Social Readjustment Rating Scale[5] (Table I) and occurring within the 6 to 12 months before enrollment. Individual scores were added together, and analysis was therefore in terms of the cumulative stress score.

Mothers were interviewed at the antenatal clinic by one of the authors (B.L.). Those eligible for entry on the basis of a history of stress, and willing to participate in the study, were randomized into support or control

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Table I. Criteria for entry[5]

Description of stress	*Score[5]*
Major marital problems	39
Severe illness in a family member	44
Dismissal from work (subject or baby's father)	47
Marriage during pregnancy	53
Significant personal injury	50
Death of a close family member (including abortion or stillbirth)	63
Imprisonment (subject or baby's father)	63
Separation from baby's father	65
Divorce	73
Death of baby's father	100

groups. Before randomization all mothers signed an informed consent form that indicated that they might or might not be selected for additional interviews or counseling sessions with a social worker involved in the study. Randomization was simple (unstratified), by means of sealed envelopes containing a green or pink slip of paper, indicating assignment to support or control groups, respectively. Mothers in the support group entered into a regular, individualized, client-centered relationship with one of two social workers contracted to the study. Both social workers were skilled counselors with experience in gestational support and crisis intervention. Support consisted of at least 20 minutes with the social worker at the time of each antenatal clinic visit. When mothers felt unable to wait for the social worker on a clinic day, arrangements were made to make up the missed visit, or for the social worker to have a 10- to 20-minute telephone counseling session with the subject. The psychosocial support was directed toward dealing with the stress factor(s) that had warranted enrollment in the study, assisting with problems in the home or work situation, and encouraging mothers to comply with instructions and advice of the antenatal clinic staff. Control mothers were treated according to the usual protocols of the antenatal clinic; those personnel, while obviously aware of the existence of our study, were largely unaware of the personal problems of the mothers.

Additional data collected in every case included maternal age, obstetric history, marital status, and number of clinic visits after enrollment. Smoking history was recorded and, while we did not include a specific antismoking program in the study design, mothers were exposed to the standard information in the form of educational posters and pamphlets at the clinic. At the time of delivery, birth weight and length were recorded for every baby by a labor ward staff member who was unaware of the subject's group assignment, and gestational age was assessed by one of the authors (A.D.R.) according to the Ballard score.[10] Mothers completing the study were interviewed within 2 weeks of delivery to assess the value of support for the study mothers and perceptions regarding need for support on the part of controls.

Statistical analysis was by means of t tests for normally distributed data or otherwise by the Mann-Whitney test; Fisher's exact or χ^2 tests were used to compare differences in proportions between groups. Interactive multiple linear regression analysis was used to explore the relationship between variables.

The study was approved by the University Committee for Research on Human Subjects.

Results

To be assured of the minimum sample size required (86 subjects, 43 per group), i.e., allowing for dropouts, abortions, and transfer of mothers to other hospitals, 104 subjects were enrolled between May 1989 and August 1990. Eight subjects were excluded (two aborted shortly after enrollment, two developed medical problems, twins were diagnosed in one, three transferred to other medical centers). Excluded subjects within each of these categories were equally distributed between support and control groups. Data collection was stopped when 43 subjects in each group had completed the study; however, the four remaining mothers in the support group continued to receive counseling until delivery, and the six remaining in the control group continued with routine care.

Table II shows the characteristics of the study population and also demonstrates how closely the present group of 86 resembles the 255 stressed mothers studied in 1985[6] in terms of maternal age, cumulative stress score, birth weight, and LBW rate. Mothers were entered into the present study at 20.7 ± 2.6 weeks of pregnancy (mean ± SD).

Interactive regression analysis of data for the total group showed that psychosocial support, gravidity, and smoking were significant determinants of birth weight, together accounting for 14.8% of the variance. Infant sex, maternal age, marital status, and previous stillbirth or abortion were not important in this model.

When the data were analyzed according to support and control groups, there was a significant reduction in the number of infants weighing <3000 gm in the supported group (7 of 43 vs 18 of 43; $p = 0.008$; odds ratio, 0.29; 95% confidence interval, 0.12 to 0.74); however, the number of LBW babies was similar in both groups (six of 43 in the supported group vs five of 43 in controls). While there was a trend toward a higher prematurity rate in the control mothers, the difference was not significant (20.5% of control mothers delivered before 37 completed weeks vs 10.8% of those who received support, $p = 0.2$). The above-mentioned weight and gestational age data suggest that psychosocial support had a greater effect on fetal weight than on du-

Table II. Major similarities between study population and 1985 group of stressed mothers[6]

	Present study (n = 86)	*Retrospective study (1985)[6] (n = 255)*
Maternal age (yr, mean ± SD)	24.5 ± 5.4	24.5 ± 5.2
Cumulative stress score (mean ± SD)	74 ± 33	72 ± 35
Birth weight (gm, mean ± SD)	3164 ± 668	3147 ± 580
LBW rate (%)	12.8	12.9

ration of pregnancy, and neonatal length and weight-to-length ratios appear to confirm this postulate. Mean neonatal length (±SD) was similar for both groups (49.3 ± 2.1 cm for control infants vs 50.0 ± 2.8 cm for those of supported mothers), while weight-to-length ratios were significantly different in the two groups (30% of controls weighed <55 gm/cm length vs 11.4% born to supported mothers; $p = 0.045$). The change in the distribution of birth weight between the groups of infants is shown in Table III.

Two neonates from the supported group required admission to the neonatal intensive care area for ventilatory support. Both were of very low birth weight and one died (875 gm). One very-low-birth-weight neonate from the control group required admission to the intensive care area for management of hypoglycemia. No neonatal problems of note were encountered in the remaining 83 infants.

Characteristics of the two groups of mothers and infants are shown in Table IV. Of particular note are the following: relatively high percentage of smokers in both groups; mean birth weight difference of 101 gm between control and supported groups; and eight or more visits to the antenatal clinic for all mothers, with supported mothers receiving seven or more sessions with the social worker in the ±18 weeks between enrollment and delivery. The significantly greater number of women with previous abortions or stillbirths in the supported group is a chance finding but even if significant would probably have acted to increase and not diminish the risk of lower birth weight in this group.[11] The effect of counseling appeared to be independent of any effect on maternal smoking; similar numbers of supported and control mothers modified their smoking habits during the study.

In Table V, mothers of infants weighing <3000 gm are compared according to support or control status. Because of the small number of infants in the support group, most of the differences are not significant; however, it would appear that the seven mothers who were "unresponsive" to support were more likely to be older, multiparous smokers who, despite a poor obstetric history, were infrequent attenders of the antenatal clinic.

Fifty-one percent of supported mothers rated the sessions with the social worker as being very helpful, 40% found the support to be of some help, 5% did not benefit at all, and 4% did not complete the questionnaire. In the control group, 58% felt that support was not needed, 30% would have appreciated some help, and 12% did not return the questionnaire. No mothers in the control group were referred for counseling by antenatal clinic staff.

Table III. Birth weight distribution of infant groups

Birth weight (gm)	*Supported (n = 43)*	*Control (n = 43)*
<2000	2	2
2000-2499	4	3
2500-2999	1	13
3000-3499	22	11
3500-3999	11	11
≥4000	3	3

Comment

Interactions between maternal behavior, inadequate social support systems, and personal maternal characteristics have been described which might lead to uteroplacental problems and premature labor.[12] In the proposed model stress leads to catecholamine release, decreased placental function, and uterine irritability. While premature labor may be the end product of stress, it is clear that uteroplacental problems might also interfere with fetal nutrition and growth. Several authors have confirmed these effects of stress in retrospective[1-4, 6] and prospective[7] studies, with most implying or concluding that randomized, controlled trials are required to establish whether the deleterious effects can be reversed by psychosocial support during pregnancy.

Elbourne[8] has commented on the data from 12 relevant controlled trials, which have been included in the Oxford Database of Perinatal Trials. In terms of the outcome measures applicable to the present study, the meta-analysis reveals that social support during pregnancy influences neither the LBW rate nor the preterm delivery rate (typical overall odds ratios, 0.89 and 0.99; 95% confidence intervals, 0.76 to 1.05 and 0.84 to 1.17, respectively).

This study differed from those reviewed by El-

Table IV. Characteristics of supported and control mothers and infants*

	Supported	*Control*	*p Value*
Maternal age (yr, mean ± SD)	25.1 ± 5.5	23.8 ± 5.3	NS
Primigravid (%)	37.2	52.3	NS
Married (%)	60.5	57.1	NS
Previous abortion or stillbirth (%)	40.5	19.0	0.027
Smoking (%)	44.2	41.5	NS
Cumulative stress score (mean ± SD)	75 ± 35	74 ± 32	NS
No. of clinic visits (mean ± SD)	8.0 ± 2.8	8.7 ± 3.0	NS
No. of sessions with social worker (mean ± SD)	7.2 ± 3.5	—	
Infant			
Weight (gm, mean ± SD)	3214 ± 649	3113 ± 690	NS
Length (cm, mean ± SD)	49.3 ± 2.1	50.0 ± 2.8	NS
Gestational age (wk, mean ± SD)	38.7 ± 3.0	38.3 ± 2.7	NS
Male/female ratio	1:1.4	1.3:1	NS
Cesarean delivery (%)	11.6	16.3	NS

NS, Not significant.

*Age, marital status, smoking, and stress score on entry into the study.

Table V. Characteristics of mothers of infants with birth weight <3000 gm

	Supported (n = 7)	*Control (n = 18)*	*p Value*
Cumulative stress score (mean ± SD)	61 ± 15	72 ± 35	0.86
No. of antenatal clinic visits (mean ± SD)	4.3 ± 2.3	7.6 ± 2.8	0.03
Age (yr, mean ± SD)	26.6 ± 7.1	23.5 ± 5.3	0.28
Primigravid (%)	14	59	NS (0.06)
Previous stillbirth or abortion (%)	57	18	NS (0.07)
Smokers (%)	71	47	NS (0.26)

bourne[8] in that we studied only white mothers subjected to moderate to severe psychosocial stress. In accordance with the meta-analysis, LBW and prematurity rates were not affected by support during pregnancy, but there was a highly significant effect on the number of infants weighing <3000 gm. Furthermore, this effect of support appeared to be due to improved intrauterine growth rather than prolongation of pregnancy. These results are reminiscent of those of Olds et al.,[13] who found that in a subset of smokers and young adolescents, intervention improved birth weight and length of gestation, but not in the lowest ranges. However, in contrast to one of the conclusions in a subsequent review by Olds and Kitzman,[14] our results were obtained by means of psychosocial support, but without specific attention to alteration of adverse behavior. Support in this study was directed toward dealing with the stress factor(s) that had warranted enrollment, and while the social workers were instructed to provide help or advice with additional domestic, occupational, or health-related problems, they were only infrequently called on to do so.

The support provided to stressed mothers in this study was rated as being very or moderately helpful by 91% of the group. In the control group 58% of mothers questioned after delivery were of the opinion that support would not have helped them, while 30% would have appreciated some assistance with their problems. In this study we did not assess other subjective aspects such as attitudes toward the pregnancy, confidence levels, or relationships with family or medical staff, preferring to use objective measurements to evaluate the effects of intervention. In this regard we did analyze the instrumental or operative delivery rates in the two groups, and, in agreement with the results of the meta-analysis,[8] found that support did not have any effect.

In evaluating the effects of a variety of stressors during pregnancy, most studies use LBW or very LBW as end points.[1-4, 7, 8] These are important measures because LBW and very LBW, whether because of preterm birth or intrauterine growth retardation, affect neonatal and postneonatal mortality, as well as subsequent infant and childhood morbidity.[15] We cannot question the rationale for assessing stress or intervention effects in terms of LBW or very LBW rates; however, we have shown that by doing so one might miss other measures of outcome, in this case the number of births between 2500 gm and 3500 gm. The significant effect of psychosocial counseling in increasing birth weight to above 3000 gm is of little consequence in terms of neonatal outcome but shows far more conclusively than before[8] that support during pregnancy has a clinically measurable effect.

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Transvaginal removal of ovaries with endoloop sutures at the time of transvaginal hysterectomy

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Twenty patients were evaluated for possible oophorectomy during transvaginal hysterectomy. Of 40 ovaries, nine were removed with a standard clamp technique. Twenty-two ovaries could not be removed by the clamp technique but were successfully removed with endoloop sutures. Safe transvaginal removal was not thought to be possible for the remaining nine ovaries. (AM J OBSTET GYNECOL 1991;165:407-8.)

Key words: Oophorectomy, transvaginal; suture, endoloop

The purpose of this study was to evaluate the feasibility and safety of transvaginal oophorectomy by the endoloop suture at the time of transvaginal hysterectomy.

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Material and methods

This was a prospective observational study. Ovarian removal by the standard clamp technique was performed when possible. If the standard technique was not feasible but the ovaries appeared to be reasonably accessible, then removal with the endoloop suture (size 0 plain gut ligature; Ethicon Inc., West Germany) was attempted. When the uterus had been removed, each ovary was grasped with a long Babcock clamp and was pulled with moderate traction into the vagina. An endoloop suture was then brought around the Babcock and was secured above the ovary at the origin of the mesovarium. A second endoloop suture was placed close to the first. The ovary was then excised under direct vision and with right-angle scissors holding the endoloop sutures.

Results

From July 1 to Dec. 21, 1990, 26 consecutive patients were eligible for this study. Six patients were excluded from the study after they declined oophorectomy; this resulted in a total of 20 patients for analysis. The mean age of the 20 patients was 50 years; the age range was 38 to 72 years. The mean parity was 2.8, with a range of 0 to 8. Five of the patients were morbidly obese. Eight patients had undergone genitourinary surgery. The primary diagnosis was symptomatic pelvic relaxation in five patients, atypical endometrial hyperplasia in three patients, grade 1 endometrial carcinoma in three patients, uterine leiomyomas in five patients, grade 3 cervical intraepithelial neoplasia in three patients, and stage IA squamous cell carcinoma of the cervix in one patient.

In four of the 20 patients bilateral oophorectomy was successfully accomplished with the clamp technique. In 10 patients bilateral oophorectomy could not be accomplished by the standard clamp technique but was successfully accomplished with the endoloop technique. In one patient one ovary was successfully removed by the clamp technique and the other ovary was removed with the endoloop technique but the other ovary was not thought to be accessible for removal. In the remaining four patients safe transvaginal removal of the ovaries was not thought to be possible and was not attempted. In the 10 patients who underwent transvaginal endoloop oophorectomy, the mean operative time (for bilateral oophorectomy) was 18 minutes, with a range of 15 to 30 minutes. The estimated blood loss for all 10 patients ranged from 0 to 5 ml.

Thirty-one ovaries were removed in this study, and 22 of these were removed with the endoloop technique. If the two largest dimensions are considered, the mean size of the ovaries removed was 3.3 × 2 cm, with a range of 1.5 to 6 × 1 to 4 cm. Of the 31 ovaries removed, four had benign pathologic findings. These findings included one fibroma, one hyperplastic stromal nodule, and two ovaries with extensive hyperthecosis. These ovaries were removed by the endoloop technique. The hyperthecotic ovaries were enlarged, and it was actually necessary to morcellate them to accurately visualize the pedicle of the mesovarium where the endoloop sutures had been placed.

There were no intraoperative complications. During the study five endoloop sutures were either broken or placed incorrectly and had to be immediately replaced. Five patients experienced postoperative febrile morbidity that resolved with intravenous antibiotics. These five patients included three who had undergone the endoloop technique, one who had undergone the clamp technique, and one in whom oophorectomy had not been attempted.

Comment

The purpose of this study was to evaluate the feasibility and safety of transvaginal oophorectomy via the endoloop technique at the time of transvaginal hysterectomy in those patients in whom the standard clamp technique was judged not feasible. Of the 40 ovaries evaluable for removal in this study, only nine were successfully removed with the standard clamp technique.[1, 2] The possibility of selection bias cannot be denied, except to say that every effort was made to avoid it. Twenty-two of the 31 ovaries thought not to be removable by the standard clamp technique were removed successfully with the endoloop technique. Also, the endoloop technique required less space and provided better visualization of the mesovarium, which may have allowed more accurate and complete removal of the ovary. Morcellation of the ovaries in one case added to visualization of the mesovarium. Morcellation of the ovaries should not be undertaken in the presence of a suspected neoplasm. The results of this study suggest that transvaginal removal of ovaries that otherwise cannot be removed by the standard clamp technique is safe and feasible in the majority of patients with the endoloop technique described.

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Improving disclosure of smoking by pregnant women

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Smoking is a major modifiable risk factor in pregnancy, and low-cost interventions have been developed and tested in diverse populations of pregnant smokers. Successful intervention depends on identification, however, and nondisclosure can be a problem. This randomized study compared rates of disclosure with two response formats—multiple choice, in which the patient is able to describe herself as having "cut down," and the usual history question, "Do you smoke?," in which she is forced to answer simply "yes" or "no." Each format was tested in both oral and written channels with a multiethnic adult prenatal population ($n = 1078$) entering care in a multispecialty group. Study results indicate that the multiple choice question improved disclosure, regardless of channel (oral versus written), by 40%. This effect was observed across racial and ethnic groups. Biochemical tests of urine samples from reported nonsmokers indicated smoking in only 3%. Eleven percent of the "nonsmokers" in the experimental groups refused consent for the urine test, however, and many of these were probably smokers. (Am J Obstet Gynecol 1991;165:409-13.)

Key words: Prenatal risk assessment, prenatal history taking, validation of self-reported smoking status

Smoking is a major modifiable risk factor in pregnancy, and recent randomized trials of low-cost counseling and self-help material tailored to pregnancy have documented increased cessation in diverse populations of pregnant smokers.[1-3] Clinically significant decreases in rates of smoking, 12 to 14 percentage points over usual care, have been achieved. Application of these intervention methods in obstetric practices, however, depends on effectively identifying smokers.

Most physicians now ask about patients' smoking habits,[4,5] and as a practical matter they must rely on simple verbal inquiry. Biochemical validation is costly, and several of the inexpensive tests are subject to substantial error in classifying individuals. Thus it is of importance that the results of recent studies evaluating the veracity of responses to verbal inquiry indicate a wide range in nondisclosure rates, from 4% to 45%.[6-9] The higher rates occur in contexts where patients perceive smoking as especially undesirable.[10] This is a likely perception for women in prenatal care, as awareness of the health risks to the fetus increases in the population.

The study reported here originated in a population-based study of a prenatal smoking cessation intervention in a health maintenance organization.[2] The investigators drew on social science methods for obtaining sensitive information[11] to develop an effective question for identifying smokers. The resulting question appeared to be advantageous, but it was not tested experimentally against alternatives. In this paper we report the results of a randomized study comparing the relative effectiveness of practical methods for identifying pregnant smokers against the traditional methods for eliciting smoking status.

Material and methods

Design. The study used a randomized, fully crossed 2×2 factorial design with two response formats ("yes" or "no" and multiple choice) and two channels of questioning (oral and written) and applied the four combinations to all eligible women on their first prenatal visit. Research literature from sociology and the previous clinical trial experience suggested that channel and response format are crucial dimensions in eliciting sensitive information. The same neutrally worded question was used in the four experimental conditions (Fig. 1).

The usual history question was represented as the dichotomous response format, whether through an oral or written channel. We selected the multiple choice format (Fig. 1) because of its success in the pilot study and its options ("I smoke every once in a while" and ". . . I have cut down since becoming pregnant") that offer the woman a means of portraying herself in a more positive light. Multiple choice may be better suited for

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Which of the following statements best describes your cigarette smoking: Would you say:

1. I smoke regularly now—about the same amount as before finding out I was pregnant.
2. I smoke regularly now, but I've cut down since I found out I was pregnant.
3. I smoke every once in a while.
4. I have quit smoking since finding out I was pregnant.
5. I wasn't smoking around the time I found out I was pregnant, and I don't currently smoke cigarettes.

Fig. 1. Multiple choice question.

Table I. Sociodemographic and obstetric history profile for eligible obstetric patients

Variable	*Total (n = 947)* (%)*
Consent for prenatal form	88.8
Smokers	89.5
Nonsmokers	88.7
Race	
White	50.1
Black	32.6
Hispanic	14.7
Other	2.7
Age (yr)	
<20	6.6
20-29	57.0
30-39	34.7
>40	1.7
Married	82.5
Gravidity	
0	25.8
1	35.0
≥2	39.4
Parity	
0	43.5
1	37.2
≥2	19.3
No. of maternal health problems†	
0	75.5
1	20.4
≥2	4.1
No. of fetal or infant health problems‡	
0	73.5
1	22.7
≥2	3.8

*Of these, 121 did not consent for use of their prenatal form.

†Maternal index score ranges from 0 to 6 and is the number of problems in this pregnancy: chronic hypertension, diabetes, heart disease, hemorrhage, pregnancy-induced hypertension, pulmonary disease.

‡Fetal-infant index score ranges from 0 to 8 and is the number of problems in previous pregnancy: mental handicap, neonatal death, neurologic disorder, prematurity, small for gestational age, sudden infant death syndrome, spontaneous abortion or ectopic pregnancy, stillbirth.

written delivery, although in a pretest all women were easily able to recall the response options after oral presentation.

The logical order of the multiple choice options is as given in Fig. 1. Although it is theoretically possible to reverse the present order, beginning the list with "I don't smoke" might program the woman's thinking to select the first and easiest alternative, thereby interfering with her consideration of a different and more honest answer. The reading level of the question was fifth grade, as rated on the Flesch-Kincaid Readability Test.

In all four experimental conditions, we combined the smoking history question with other questions about personal behavior to form a presumed gradient of sensitivity. Thus use of automobile safety belts preceded the smoking question, and questions about alcohol and illicit drugs followed it. The format of the alcohol question was always identical to the smoking question to maintain a plausible flow of questions, and the ordering of the four questions in each set was constant.

Within each of the four clinic sites all women seen on a given day were randomly assigned to one of the four experimental conditions. This clustering of one to 10 women who might be seen in one day's intake was to assure integrity of application and to fit the project into the clinic routine as smoothly as possible.

Population. All women ≥18 years old were eligible for the study if they were able to speak and read English, were free of mental and sensory handicaps, and made their first prenatal visit at one of four multispecialty group satellite clinic locations during a 17-month period (September 1988 through February 1990). Ability to read and speak English was established with a single question, "Do you speak and read English?" We excluded potential subjects if a sensory handicap or evidence of mental retardation or mental illness was found in the course of the history and education session with the nurse educator.

Procedures. The experiment was a part of the first visit for prenatal care after pregnancy had been confirmed. Prenatal intake visits were scheduled on a single day of the week. At this visit all women saw a specially trained nurse educator to give their medical history, discuss clinic procedures, and receive advice about diet, smoking, alcohol and other drug use, and warning signs of problems in the pregnancy. The sessions lasted approximately 40 minutes. The patient then gave urine and blood samples. Over the course of the study, 19 nurse educators conducted sessions in the four clinics, with a range of two to 203 sessions per nurse, with five nurses doing fewer than 25 sessions and four doing 99 or more each.

The experimental questions were embedded in the history questions and were substituted for usual ques-

Table II. Disclosure of current smoking by experimental condition

	Multiple choice		*Dichotomous*		*Total*	
	%	*No.*	*%*	*No.*	*%*	*No.*
Oral	14.34	36/251	8.56	22/257	11.4	58/508
Written	13.60	37/272	9.73	29/298	11.6	66/570
TOTAL	13.95	73/523	9.20	51/555	11.5	124/1078

tions about smoking, alcohol, and illicit drugs. The history already contained both oral and written components, so the experimental variation was easily integrated.

Self-report of sensitive behavior can be influenced by the knowledge that an objective measure of the same behavior will be used to verify the report.[12] Thus only after the history and education components of the session were completed and before being directed to the laboratory were all eligible women asked to give written consent for copies of their prenatal history forms to be used anonymously by the university-based researchers. Those women who said they were not smoking currently also were asked for written consent to conduct an additional test on the urine sample they would be giving at the laboratory. The same procedure was used for all four groups so that knowledge of the test did not influence response to the smoking history questions. The test was described as (1) anonymous and (2) "a test for by-products of exposure to cigarette smoke in pregnant women." This procedure was approved by the human subjects protection committees at the university and the clinic. Except for a study number, identifying information was removed from the history forms and urine samples before being transferred to the researchers.

Validation of self-report. The urine samples of all women who reported nonsmoking and who gave consent for the test were analyzed for presence of a major nicotine metabolite, cotinine. This assessment was by enzyme immunoassay,[13] with the laboratory blinded as to subjects' group assignment. The sensitivity of this test is 93%, with a specificity of 95% for serum in pregnant women.[14] All recommended precautions were observed in labeling, handling, storing, and shipping the urine samples. Forty-eight samples were cross-validated with liquid gas chromatography analysis, a more sensitive and specific procedure.[15, 16] There was 100% agreement on both positive and negative samples, with a cutoff of ≥50 ng/ml.

Analysis. We analyzed the data by means of a logistic regression model. The primary dependent variable was self-reported smoking on either the dichotomous question or a "yes" response to any of the first three categories on the multiple choice question. The independent variables were response format, dichotomous or multiple choice, and channel of question, oral or written. Interaction of the two also was tested.

An alternative measure of the extent of smoking disclosure was developed from the giving or withholding of informed consent to allow a urine analysis. Those patients who said they were not smoking but refused to give consent were classified as disclosures. This measure excluded those patients who admitted to smoking and thus potentially identified another population, i.e., those who would not disclose smoking behavior regardless of the format and channel of questioning.

Results

Of 1206 pregnant women seen for care during the study period, 1078 (89.4%) were eligible for the study. The two major reasons for ineligibility were being <18 years old (37%) and not speaking and reading English (24%). Sociodemographic and obstetric history profiles are given for each experimental group and for the total sample, excluding 121 women who did not give consent for use of their prenatal history form (Table I). The sample can be described as representing white, black, and hispanic ethnic and racial groups, married, having a mean age of 28 years, with one or fewer living children, and with the large majority having no maternal or previous fetal or infant health problems. The nurses frequently omitted questions about maternal and paternal schooling, and thus these characteristics are not known. Random assignment appears to have yielded equivalent groups on measured variables.

The results of the analysis regarding self-reported smoking showed disclosure was affected by format rather than channel of questioning. More women reported smoking as a function of being asked using the multiple choice format than being asked using the dichotomous format ($p < 0.014$). The channel of questioning, oral or written, did not result in different levels of self report, and there was no interaction of format and channel.

Table II provides the frequencies of reported smoking related to format. The multiple choice format elicited a five percentage point higher (13.96% versus 9.19%) positive smoking response (odds ratio, 1.60; 95% confidence interval, 1.10 to 2.33). There were no differences among ethnic or racial groups. Within the multiple choice question, distribution of responses indicating current smoking was as follows: cut back for the pregnancy, 61.6%; smoke every once in a while, 21.9%; and smoke the same amount as before pregnancy, 16.4%.

The second phase of the study tested withholding of consent for the additional urine test as a measure of nondisclosure in response to the questions about smoking. The same categorical model was applied to the urine consent measure for 954 self-reported nonsmokers. No interaction effects or main effects were found for format and channel. The overall rate of nonconsent was 11.0%. The urine test results indicated smoking in fewer than 3% of those who gave consent for the test.

Comment

The 40% improvement in disclosure found in this study is statistically and clinically significant. It was observed across three ethnic and racial groups in a clinical context where patients gave their responses without being influenced by knowledge of the research. Also, the questions were tested by interviewers with a range of backgrounds and interviewing skills.

The lack of difference between the oral and written formats suggests that the written form could be used by any health care provider. Because the "demand" to give a desirable answer to a physician may be higher than to a nurse and because of the time savings, we recommend use of the written format.

It is probable that the current association of urine testing with screening for illicit drugs produced some of the nonconsent for the urine tests. Nevertheless, many of the 11% who did not give consent for the urine test undoubtedly are smokers. This group of unidentified smokers suggests the limits on the sensitivity of the history-taking method tested here.

The difference in disclosure that we found, however, would mean that over 55,000 additional pregnant smokers could be identified annually by simple changes in history taking. These identification methods did not elicit a defensive reaction by the woman, as would other means such as nonvoluntary biochemical testing, and the multiple choice question may give more information about the patient's smoking habit. The response option most frequently selected by current smokers, having cut down since the pregnancy began, appears to reflect the true behavior in many women early in pregnancy. For this group and others who had not really cut down, this response offers a means of displaying a partially positive image to the prenatal caregiver. Thus the additional women identified are expected to be as amenable to intervention as others. Such intervention should be a high priority in prenatal care not only to help prevent low birth weight[17, 18] but also to reduce exposure to environmental smoke by infants[19, 20] and to reduce smoking among younger women whose rates of smoking make them a high priority target for smoking cessation efforts.[21-24]

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Thrombotic thrombocytopenic purpura first seen as massive vaginal necrosis

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Thrombotic thrombocytopenic purpura is a hematologic disorder that affects the microcirculation. A 38-year-old woman was first seen with fever, thrombocytopenia, and vaginal bleeding. Pelvic examination revealed massive vaginal necrosis. Hematology consultation resulted in agreement with the diagnosis of thrombotic thrombocytopenic purpura. This is the first reported case of thrombotic thrombocytopenic purpura first seen as vaginal necrosis of which we are aware. (Am J Obstet Gynecol 1991;165:413-5.)

Key words: Thrombotic thrombocytopenic purpura, vaginal necrosis, cocaine

Thrombotic thrombocytopenic purpura is a rare, life-threatening disorder characterized by the pentad of fever, microangiopathic hemolytic anemia, thrombocytopenia, neurologic symptoms, and renal dysfunction. The pathogenesis of thrombotic thrombocytopenic purpura, however, remains unclear. In >90% of cases, purpura is the initial manifestation and may be associated with epistaxis, hematuria, gastrointestinal hemorrhage, menorrhagia, and hemoptysis. Fever is noted in all cases.[1, 2] The following represents the first known case of thrombotic thrombocytopenic purpura first seen as massive vaginal necrosis of which we are aware.

Case report

A 38-year-old gravida 3, para 3 black woman went to a local emergency room with a 3-day history of fever, chills, abdominal pain, vaginal bleeding, and discharge. Examination was remarkable only for mild abdominal tenderness. Pelvic examination was refused because of discomfort. Vital signs were stable. Laboratory data revealed a thrombocytopenia of 42,000/mm^3. She refused admission. The next day her symptoms worsened and she returned to the emer-

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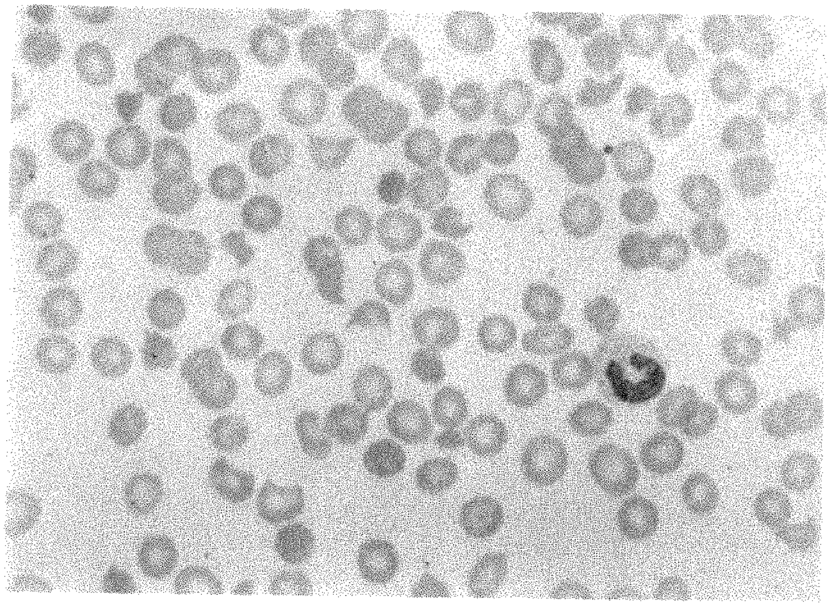

Fig. 1. Peripheral blood smear shows red blood cell fragmentation.

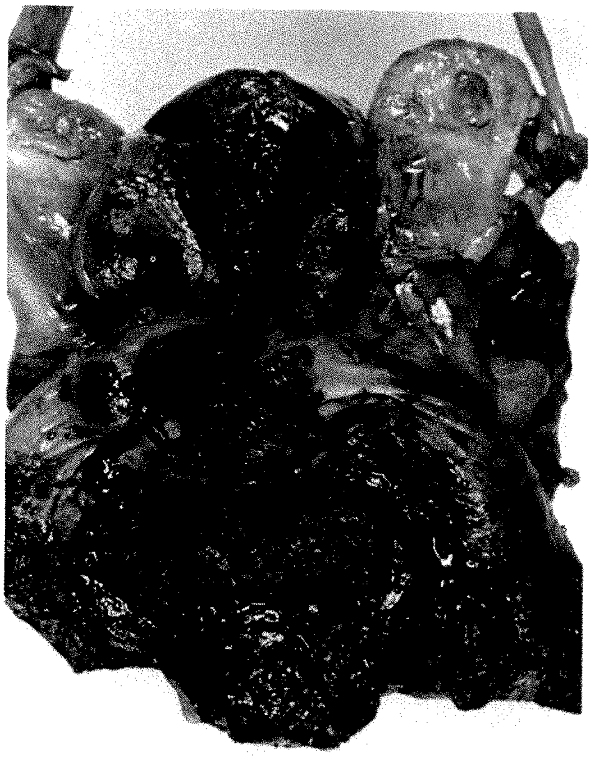

Fig. 2. Extensive necrosis and hemorrhage of the vagina.

gency room. Examination results were unchanged. White cell count was within normal limits. Platelet count was 8000/mm^3 and the hemoglobin value had dropped from 13.4 to 8.6 gm/dl. The coagulation profiles were normal. Blood urea nitrogen and creatinine values had risen from normal to 66 mg/dl and 3.2 mg/dl, respectively. The drug screen was positive for cocaine. She was admitted to the local hospital with the presumptive diagnosis of pelvic inflammatory disease and sepsis complicated by disseminated intravascular coagulation.

Treatment was begun with platelet and blood transfusions and antibiotics (clindamycin, aztreonam, and ceftriaxone sodium). Computed tomography scan of the abdomen showed an enlarged uterus with a bacteriogenic gas pattern, suspicious of uterine abscess versus degenerative malignant changes. Cultures of blood, urine, and cervix were without growth. Hematemesis developed 48 hours after admission and she was transferred to the Medical College of Georgia.

Pelvic examination at the time of admission to the Medical College of Georgia revealed proximal vaginal necrosis with sloughing of tissue. Examination after induction of anesthesia, dilatation and curettage, and biopsies were done for a more thorough evaluation. A circumferential vaginal lesion extended anteriorly throughout the entire length of the vagina with minimal posterior involvement. The tissue was dark red, nonnodular and friable. No cervical lesions were noted. The uterus sounded to 8 cm. Histopathologic diagnoses included vaginal necrotic debris, chronic cervicitis with acute hemorrhage, and proliferative endometrium.

Hematology consultation was obtained. A diagnosis of thrombotic thrombocytopenic purpura was made on the basis of the peripheral blood smear (Fig. 1), thrombocytopenia, fluctuating mental status, renal dysfunction, and fever. Treatment was begun with four units of fresh frozen plasma every 6 hours until plasma exchange could be instituted the next morning. That evening, 2 days after admission, she became lethargic, apneic, and died of a cardiopulmonary arrest.

At autopsy, numerous petechial hemorrhages of the

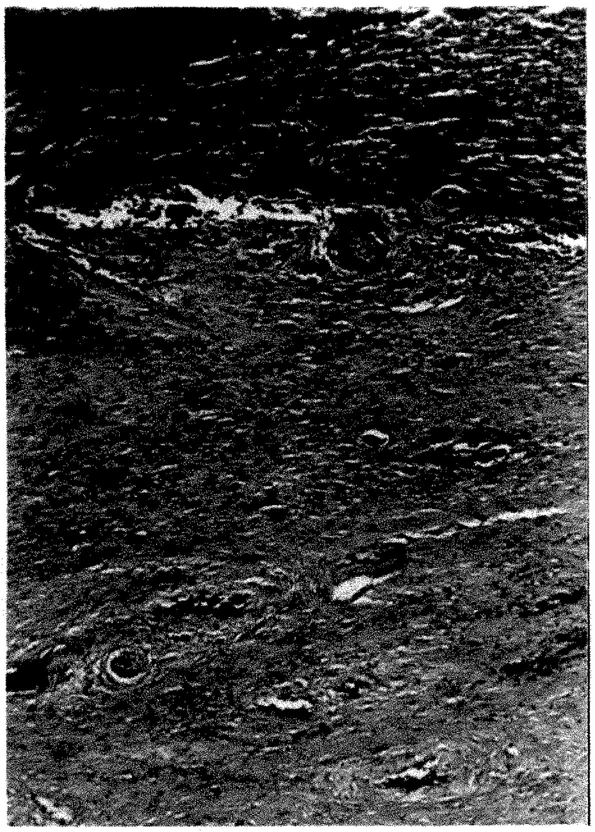

Fig. 3. Numerous microscopic arteriolar thrombi in vaginal wall.

endocardium, myocardium, epicardium of the heart, the pleurae of lungs, kidneys, intestines, liver, and pancreas were noted. Extensive necrosis and hemorrhage of the cervix and vagina were seen (Fig. 2). Numerous microscopic arteriolar thrombi were identified in the heart, lungs, kidneys, gastrointestinal tract, liver, pancreas, wall of the vagina (Fig. 3), and urinary bladder. Local hemorrhage, blood pooling, hemosiderosis, hemophagocytosis, and subendothelial abnormalities were also present.

Two important historic facts were elucidated after the death and autopsy of our patient. First, before her transfer her boyfriend had an influenza-like syndrome with a 2-week history of high fever before seeking medical attention. En route to the hospital, he had a cardiopulmonary arrest and was resuscitated. He died within 24 hours of his admission with a coagulopathy and renal failure with anuria. An autopsy was denied. Second, the patient's mother wrote a letter to the medical records department at the hospital and stated that her daughter recently acknowledged someone had attempted to poison her. A telephone conversation with the patient's mother also revealed a 2-year history of crack cocaine abuse by the patient and the patient's boyfriend.

Comment

The diagnosis of thrombotic thrombocytopenic purpura was confirmed histologically at autopsy. The concomitant findings of arteriolar thrombi, hyaline subendothelial deposits, periarteriolar concentric fibrosis, hemophagocytosis, and hemosiderosis are consistent with the diagnosis of thrombotic thrombocytopenic purpura. Arteriolar thrombi are noted in 75% of thrombotic thrombocytopenic purpura cases.[1] The spleen had numerous periodic acid-Schiff–positive, subendothelial deposits, a common characteristic seen in most cases of thrombotic thrombocytopenic purpura.[2] Hemosiderosis and hemophagocytosis were identified in the spleen, liver, and lungs, a finding in 83% of affected patients. Periarteriolar concentric fibrosis was seen in the spleen and is reported in 58% of patients.[1]

In our case, the history of cocaine abuse, possible poisoning, and the simultaneous illness and subsequent death of her partner suggest a common exposure to an environmental substance as the cause of the syndrome. Reports in the literature that implicate cocaine as an etiologic factor are not noted. However, the cocaine or a contaminant in the crack cocaine could be responsible for triggering the syndrome.

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Association of immunoreactive eosinophil major basic protein with placental septa and cysts

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A protein that is immunochemically indistinguishable from the major basic protein of the eosinophil granule is present at elevated concentrations in the plasma of pregnant women. Major basic protein has been localized to placental trophoblasts known as X cells. Because X cells are located in placental septa and septal cysts, we tested whether the numbers of these structures are correlated with plasma levels of major basic protein. Data analysis revealed strong positive correlations between maternal plasma major basic protein concentrations and the number of placental septa, septal cysts, and subchorial cysts and the presence of subchorial fibrin deposits. No significant correlation was found between plasma major basic protein levels and other variables examined, such as placental weight. Thus major basic protein is a specific marker for septa and cysts, presumably because it is an X-cell product. The study of major basic protein may aid in delineating the function and ontogeny of X cells, as well as their role in fibrin deposition and in septa and cyst formation. (AM J OBSTET GYNECOL 1991;165:416-20.)

Key words: Pregnancy, major basic protein, placental septa, eosinophil, placental cysts

A protein identified immunochemically as the major basic protein of eosinophil granules is present in the plasma of pregnant women at elevated levels ranging from 750 to 8000 ng/ml (nonpregnant, <600 ng/ml).[1] Major basic protein has been localized to placental trophoblastic cells known as X cells.[2] We hypothesized that the X-cell mass would be related to the major basic protein level in the maternal plasma. X cells line septal and subchorial cysts and are found in the cell columns of anchoring villi, in septa, and as cell islands in the basal plate.[3-13] The number of grossly visible septa and septal cysts varies widely from placenta to placenta.

We have quantified the presence of these structures as a measure of X-cell mass to determine whether this mass is related to maternal plasma major basic protein level.

Methods

Placentas were obtained at delivery and stored at 4° C. Surface features examined were cord length, insertion, and vasculature; membrane integrity; presence of subchorial fibrin; and the number of subchorial cysts. The weight and dimensions were recorded after cord and membranes were removed.

Three hundred seventy-nine placentas were examined and the gross morphologic types of each were recorded. In 80 cases plasma was obtained in ethylenediaminetetraacetate from the same women during labor. The plasma was stored at −20° C until subjected to radioimmunoassay for major basic protein as described.[14] Patient histories were reviewed for information regarding weeks of gestation at delivery and gestational complications. The following complications were noted: preeclampsia ($n = 6$, 1.6%), hypertension ($n = 3$, 0.8%), intrauterine growth retardation ($n = 4$, 1.1%), twin gestation ($n = 5$ from three sets of twins, 0.01%), intrauterine fetal death ($n = 1$, 0.3%), and preterm delivery at <37 weeks' gestation ($n = 29$, 7.7%).

Serial sections were cut from the maternal surface to the chorionic membrane to quantitate the total number of septa and septal cysts, the degree of calcification, and the presence of infarcts. Sections were cut at 1 cm intervals by single strokes of a large knife. The cut was exposed by pressing the tissue back with the knife, and the total number of septa seen was recorded. The number of septa seen for each cut was summed to generate an index of the total number of septa per placenta. Septa were recognized as thin, translucent, membranous structures with a grayish hue that extended from the maternal face toward the chorionic membrane. Two septal indices were devised to compensate for the sampling error caused by differences in size of the placentas: (1) total number of septa per number of sections

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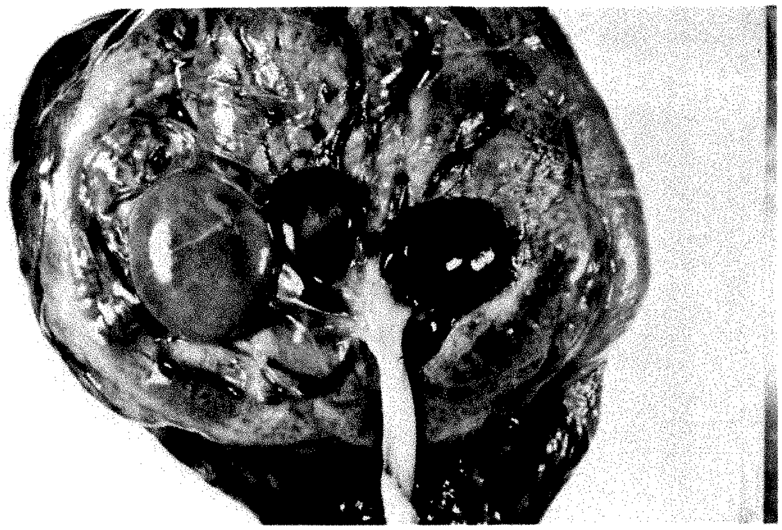

Fig. 1. Placenta containing three subchorial cysts.

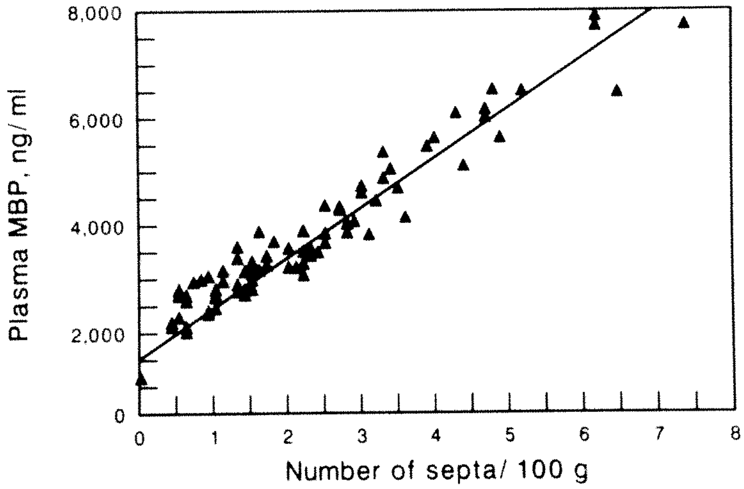

Fig. 2. Relationship between placental septa and plasma major basic protein *(MBP)* ($r = +0.96$; $p < 0.001$). This figure includes observations on all 80 patients who had plasma major basic protein measurements, including those six with complications of pregnancy.

cut and (2) total number of septa/100 gm of placental weight.

Results

In eight of the 379 placentas, no septa were visible macroscopically. In placentas containing numerous septa, these structures often showed signs of degeneration—presence of perforations, fibrin, calcium deposition, and cysts. Cysts associated with septa ranged from 1 mm to 1 cm in diameter and were filled with a clear mucinous fluid. Macroscopic septal cysts were seen in 92 placentas (24%). Subchorial cysts varied from 5 mm to 6 cm in diameter and were present in 28 placentas (7%). One placenta had three large cysts (Fig. 1) that yielded 30 ml of a straw-colored, mucinous fluid containing major basic protein at 25 μg/ml. The incidence of cysts reported here is in agreement with observations by other authors.[3, 4]

Spearman rank correlation analysis and regression analysis were used to compare the anatomic features of placentas with plasma major basic protein levels in 74 subjects. This group includes those with a plasma sample drawn during labor and does not include those with the gestational complications previously mentioned (six patients). Spearman correlation coefficients are reported in parentheses along with associated

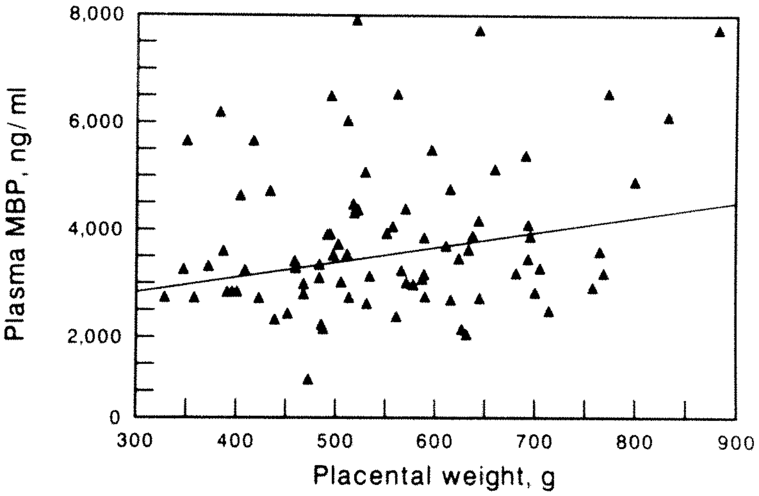

Fig. 3. Relationship between placental weight and plasma major basic protein *(MBP)* (r = 0.2; not significant). This figure includes observations on all 80 patients who had plasma major basic protein measurements, including those six with complications of pregnancy.

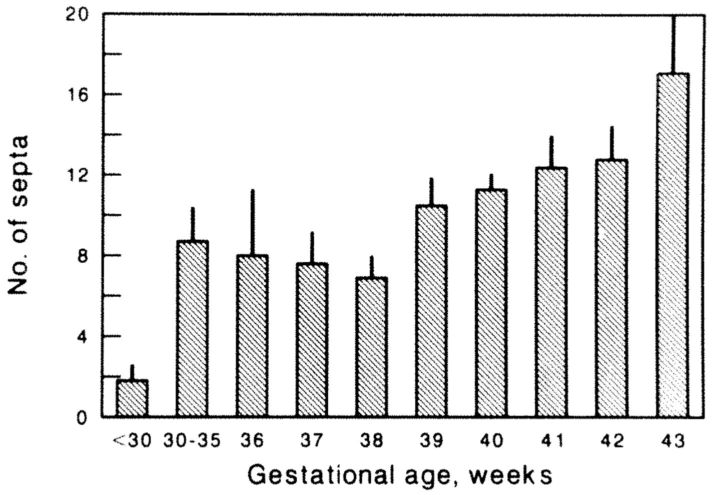

Fig. 4. Relationship between gestational age and placental septa, shown as mean ± SE. For difference between mean number of septa at <30 weeks and at 38 weeks, $p < 0.01$; for difference between mean number of septa at 38 weeks and at 43 weeks, $p < 0.001$.

p values. Plasma major basic protein level was correlated with the total number of septa ($r_s = 0.91, p < 0.0001$), number of septa per section ($r_s = 0.92$, $p < 0.0001$), and number of septa per 100 gm of placental weight ($r_s = 0.96, p < 0.0001$) (Fig. 2). Plasma major basic protein levels also were correlated with the number of septal cysts ($r_s = 0.55$, $p < 0.0001$), subchorial cysts ($r_s = 0.26, p < 0.05$), and presence of subchorial fibrin plaques ($r_s = 0.33$, $p < 0.01$). Unlike the situation reported for many placental proteins,[15] the plasma major basic protein level was not related to the weight of the placenta ($r_s = 0.21, p < 0.05$) (Fig. 3). Major basic protein concentration was not related to any of the other placental variables considered or to fetal gender, fetal weight, maternal age, or maternal parity.

There is an increased number of grossly visible septa in gestationally more mature placentas (Fig. 4).

Comment

Plasma levels of major basic protein in individual pregnant women can vary from 750 to 8000 ng/ml.[1] This study demonstrates that the plasma level of major basic protein is directly correlated with the presence of septa, septal cysts, and subchorial cysts in the placenta. Because these structures contain large numbers of X cells, this suggests that the mass of these trophoblastic cells determines maternal blood levels of major basic protein. This premise is supported by the finding that fluid aspirated from septal and subchorial cysts contains large amounts of major basic protein (up to 100 μg/ml).[2] Furthermore, placental extracts made from septal tissue contain 10 times as much major basic protein per gram of tissue as does whole placenta (unpublished data).

The possibility that major basic protein is a product of X cells has important implications. First, as a specific protein marker for X cells, major basic protein could be useful for defining the poorly understood origin and developmental pathways of these unusual trophoblastic cells.[3, 6, 10-13, 16, 17] For example, the finding that major basic protein is present in placental site giant cells[2] is the first suggestion of a relationship of these cells to X cells. Second, investigation of the biologic properties of pregnancy major basic protein may provide important insights into the poorly understood function of these cells.[3-13, 16, 17] Eosinophil major basic protein, a highly basic protein (isoelectric point > 10),[18] affects clotting,[19] is a potent and unique platelet agonist,[20] and is cytotoxic to parasites and mammalian cells.[18, 21]

We believe that the increase in septa in more mature placentas does not reflect the generation of new septa but rather does reflect a maturation of septa so that they are more clearly visible later in gestation. This finding is supported by ultrasonographic studies of placentas during gestation; echo-free areas representing calcium and fibrin deposits in septa are increasingly apparent from 36 weeks to term, and the appearance of these enlarging anechoic spaces is correlated with mature lecithin/sphingomyelin ratios.[22] Therefore specific changes occur in placental septa that reflect fetal maturity. We believe that this maturation process involves, in addition to fibrin and calcium deposition, cellular degeneration, which is reflected by the presence of septal cysts[12] (cysts were not seen at <35 weeks' gestation). In light of the correlations reported here, major basic protein is implicated as a protein marker for these changes. This is supported by the discovery of a sharp rise in maternal plasma major basic protein levels from 36 weeks to term in most pregnancies.[23]

Recent work has shown that the major basic protein molecule that is extractable from placenta and from septal cyst fluid is essentially indistinguishable from the eosinophil granule major basic protein.[24] Production of a major basic protein cytotoxic molecule by X cells could explain the ability of these cells to invade decidua,[9, 11, 25] the formation of septal and subchorial cysts,[3, 4, 12] and the role of X cells in fibrin deposition.[9, 20, 25] In addition, the study of pregnancy major basic protein may further our understanding of the mechanism of septum formation and the physiologic importance of these structures, which is still in doubt.[3, 4, 16, 17, 23] Finally, because X cells are in intimate contact with maternal decidua, the study of X cell products such as major basic protein may provide insights into the mechanism of fetal allograft maintenance.

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A case report of massive vulvar edema during tocolysis of preterm labor

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Bilateral vulvar edema that is not associated with preeclampsia has been reported only rarely during pregnancy or the puerperium. A primiparous patient in premature labor at 34 weeks' gestation with no history of lymphatic or venous obstruction underwent combination tocolysis with intravenous ritodrine and magnesium sulfate. On the fifth day of tocolysis, edema developed in the right labium majus pudendi and gradually spread to the left labia during the ensuing 24 hours. The patient remained afebrile, normotensive, and without signs of localized infection or anasarca. A trial of triple intravenous antibiotics and local skin care proved ineffective. By the ninth hospital day, the edema and discomfort had progressed, thereby precluding vaginal examination without sedation. In spite of progressive cervical dilatation with tocolysis, cesarean delivery was performed. With no further treatment, the vulvar edema gradually resolved during the next week. (AM J OBSTET GYNECOL 1991;165:420-2.)

Key words: Edema, vulvar, bilateral; preeclampsia; tocolysis

A case of bilateral vulvar edema during tocolysis is described.

Case report

A 20-year-old woman, para 1-0-0-1, who did not have prenatal care, presented for evaluation of premature labor 34 weeks after her last menses. She complained of regular contractions but denied vaginal bleeding or rupture of the membranes. Her first pregnancy had resulted in an uncomplicated term vaginal delivery. Physical examination was remarkable for a cervix that had dilated to 3 cm, was 50% effaced, and had a vertex at the −2 station. Ultrasonography was significant for a normal-appearing fetus with an estimated weight of 1980 gm and measurements consistent with 34 weeks' gestation. External fetal heart rate monitoring demonstrated a reassuring fetal heart tracing and contractions every 6 minutes. Laboratory values included a hematocrit of 29%, an O-positive blood type, a nonreactive rapid plasma reagin test, and urine that was leukocyte esterase positive. Two subcutaneous injections of 0.25 mg terbutaline failed to stop the contractions. Therefore intravenous magnesium sulfate was administered for tocolysis. An amniocentesis revealed a lecithin/sphingomyelin ratio of 1.8 : 1 and absence of phosphatidylglycerol. Amniotic fluid and cervical cultures were negative. Because of fetal immaturity, intravenous tocolysis was continued. In addition, intravenous cephapirin sodium was administered for a presumptive diagnosis of cystitis. Magnesium sulfate was

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6/1/29549

discontinued because of areflexia, and intravenous ritodrine was started. With the progression of premature labor, low-dose intravenous magnesium sulfate was reinstituted. She continued to have irregular contractions, but there was no further cervical change. On the fourth hospital day the right labia were edematous. There was no evidence of erythema or induration.

During the patient's hospitalization of 8 days, her daily liquid intake exceeded her urinary output by approximately 700 ml per day. She remained normotensive, and skin sensitivity testing failed to reveal evidence of atopy. An empiric trial of nafcillin, gentamycin, and clindamycin was initiated. In spite of additional treatment with ice packs, the edema progressed, involving both labia and the lower extremities by the second hospital day.

On the sixth hospital day, intravenous tocolytics had been discontinued, and oral terbutaline was started. Repeat laboratory values were unremarkable, with the exception of a hematocrit of 22%. Simultaneous values for calcium and albumin were also low, at 8.0 and 2.7 mg/dl, respectively. The patient was anemic and received 2 units of packed red blood cells. By the eighth day of hospitalization, the labial edema had progressed to the point of occluding the vaginal outlet, and cervical examinations could not be performed (Fig. 1). Without evidence of cause, lymphatic occlusion that resulted from an enlarged uterus was thought to be the underlying factor. Because of the resumption of uterine contractions, the inability to assess cervical dilation, and the fear of marked vulvar trauma as a result of vaginal delivery, a primary low-transverse cesarean delivery was performed. A viable 2320 gm female infant was delivered. No edema was noted in the pelvis at the time of operation. The postoperative course was unremarkable, and the vulvar edema spontaneously resolved during the subsequent 4 days.

Comment

Vulvar edema can be an allergic manifestation or can result from a local insult caused by trauma, infection, or vascular or lymphatic obstruction. Vulvar edema has also been associated with systemic disease states that commonly produce pedal edema. In nonpregnant patients these conditions include malnutrition, anemia, congestive heart failure, renal failure, hepatic failure, and malignancies.

Pregnancy complications associated with vulvar edema include preeclampsia and a prolonged second stage of labor. Generalized edema in preeclampsia is usually most pronounced in the dependent areas of the body. Swelling from a prolonged second stage usually occurs after delivery. In addition, the use of the birthing chair has been reported to produce labial edema[1]; both the dependent position and an increased hydrostatic pressure may contribute to the swelling.

In our patient there was no history of or clinical evidence of trauma. Although antibiotics were given empirically, we do not believe that the edema was the result of an infection or was an allergic reaction. The patient did not use a birthing chair, but while she was at bed rest the labia were the most dependent parts of her body.

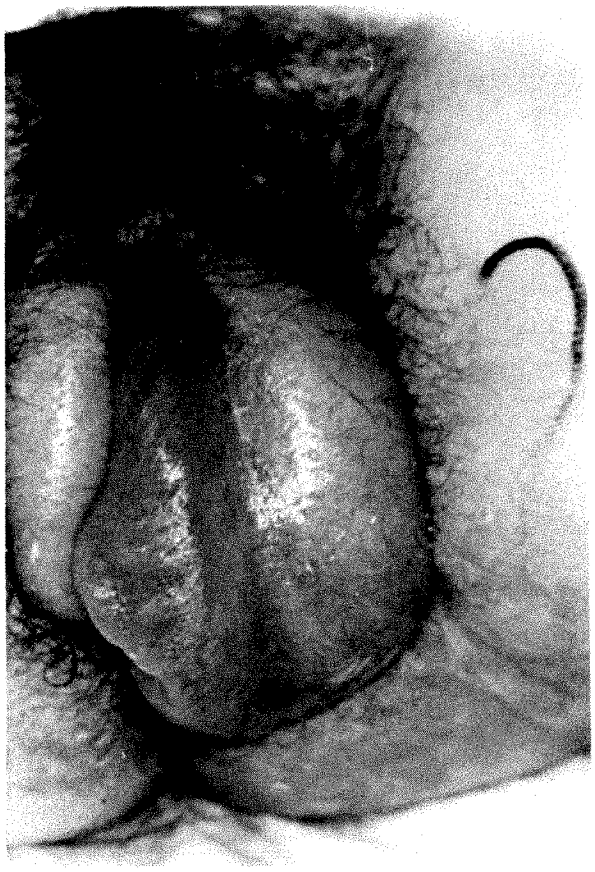

Fig. 1. Edematous labia on hospital day 8.

Fluid retention is a known side effect of intravenous tocolytic therapy, and pulmonary edema is a serious complication of this retention.[2] The fluid retention that accompanies the administration of tocolytics has been attributed to the β-adrenergic receptor agonist effect on the renin-aldosterone system and arginine vasopressin level.[3] Sodium and water retention occurs with subsequent increased hydrostatic pressure and decreased oncotic pressure.[3] The decreased hematocrit without blood loss or hemolysis is consistent with fluid retention and an expanded intravascular blood volume.

There have been several case reports of postoperative fluid shifts that resulted in unilateral or bilateral vulvar edema after the administration of intraperitoneal Hyskon contrast medium. This observation supports the hypothesis of fluid retention from tocolytics as the cause of the vulvar edema in this particular patient.

We postulate that the pathophysiologic characteristics of our case of vulvar edema involved a combination of fluid retention with resultant volume overload, decreased oncotic pressure, and increased hydrostatic pressure that resulted in a net fluid shift from the intravascular to extravascular tissues. This edema was probably most pronounced in the labia because of their dependent nature that was caused by bed rest. We believe that the cause of the fluid retention was a complication of the administration of tocolytic agents.

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A complete list of references is available from the authors on request.

Uterine activity after preterm premature rupture of the membranes

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Preterm premature rupture of the membranes complicates few pregnancies but is a major contributor to overall perinatal morbidity and mortality. Although a reduced incidence of preterm premature rupture of fetal membranes has been reported in women who had antepartum uterine activity monitoring, there are few data regarding uterine activity after preterm premature rupture of fetal membranes. Therefore daily uterine activity monitoring was performed in 101 consecutive women with preterm premature rupture of fetal membranes between 26 and 34 weeks' gestation. The mean gestational ages at rupture and delivery were 31.4 ± 2.3 and 33.7 ± 4.5 weeks, respectively. A significant increase in contraction frequency was identified within 24 hours of onset of preterm labor ($p < 0.005$). A contraction frequency of four or more per hour predicted the onset of labor within 24 hours with a sensitivity of 72%, a specificity of 90%, a positive predictive value of 54%, and a negative predictive value of 95%. These results indicate that most women with preterm premature rupture of fetal membranes exhibit a baseline contraction frequency that is similar to that of women with intact membranes and premature labor. An abrupt increase in contraction frequency is a warning of impending labor. (AM J OBSTET GYNECOL 1991;165:422-5.)

Key words: Preterm premature rupture of the membranes, uterine contraction frequency

Although preterm premature rupture of the membranes is an infrequent complication of pregnancy, it contributes significantly to perinatal morbidity and mortality.[1] The most common sequela is respiratory distress syndrome, which occurs in 10% to 40% of preterm infants whose mothers had premature rupture of fetal membranes. Other possible complications include neonatal sepsis, skeletal malformations, pulmonary hypoplasia, abnormal presentations, and cord prolapse. The perinatal mortality associated with preterm premature rupture of fetal membranes has been reported to range as high as 20% to 50%.[2] The cause of preterm premature rupture of fetal membranes is unknown but is likely to be multifactorial. Preterm premature rupture of fetal membranes has been associated with uterine overdistention, cervical incompetence, malpresentation, asymptomatic genital tract infection, and local defects within the membranes.[3-7] Subclinical preterm labor also may have a role in preterm premature rupture of fetal membranes, but no direct supporting evidence exists. This study assesses daily uterine activity monitoring in a large cohort of women with expectant management after preterm premature rupture of fetal membranes. This study was performed to evaluate the possible relationship of preterm premature rupture of fetal membranes and increased uterine activity.

Material and methods

A prospective evaluation of daily uterine activity was performed in 101 consecutive women with preterm

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premature rupture of fetal membranes who were between 26 and 34 weeks' gestation. Patients were excluded if there was evidence of active labor, chorioamnionitis, fetal distress, or fetal lung maturity during an initial 24-hour observation period.

During the initial observation, membrane rupture was confirmed by Nitrazine paper testing, ferning of vaginal fluid, and ultrasonographic evaluation of amniotic fluid volume. A visual assessment of cervical dilatation and effacement was performed, and a cervicovaginal culture for group B streptococcus was obtained. Each patient was given antibiotics pending the culture results. Tocolysis was used only during the initial 24 hours. Betamethasone (12 mg intramuscularly, 24 hours apart) was optional in gestations of <30 weeks.

Expectant management included hospitalization with bed rest, no tocolytic therapy for spontaneous labor, twice-daily patient assessment, and twice-weekly fetal assessment. Each patient performed daily uterine activity monitoring from 7 to 9 AM. The patients and clinicians were blinded to the objective uterine activity data. Delivery was allowed for spontaneous labor, chorioamnionitis, fetal pulmonary maturity, or other maternal or fetal indications.

Results are expressed as mean ± SD, and statistical analysis was performed with the Student *t* test and analysis of variance for repeated measures.

Results

Of the women with preterm premature rupture of fetal membranes who were between 26 and 34 weeks' gestation and who were evaluated at our institution between July 1987 and October 1988, 65% ($n = 101$) were candidates for expectant management. The mean patient age was 24.2 ± 5.7 years, and the mean gravidity and parity were 2.8 ± 1.8 and 1.3 ± 1.6, respectively. The mean gestational age at premature rupture of fetal membranes was 31.4 ± 2.9 weeks, and the mean gestational age at delivery was 33.7 ± 4.5 weeks. The latent period for women qualifying for expectant management was 5.3 ± 2.7 days. Indications for delivery included spontaneous labor ($n = 79$), chorioamnionitis ($n = 8$),and maternal-fetal indications ($n = 14$).

The hourly contraction frequency for each day preceding the onset of labor in those women with spontaneous labor after preterm premature rupture of fetal membranes is presented in Fig. 1. There is no significant difference between this uterine activity pattern and that previously reported (Fig. 2) for a group of women with spontaneous preterm labor and intact membranes.[7]

Comment

Despite a significant contribution to perinatal morbidity and mortality, the cause of preterm premature rupture of the membranes remains unknown. Many factors are thought to play a role in preterm premature rupture of fetal membranes, including genital tract infections, uterine overdistention, abnormalities of the internal cervical os, and excessive subclinical uterine activity. This study attempts to address the relationship between uterine activity and preterm premature rupture of fetal membranes.

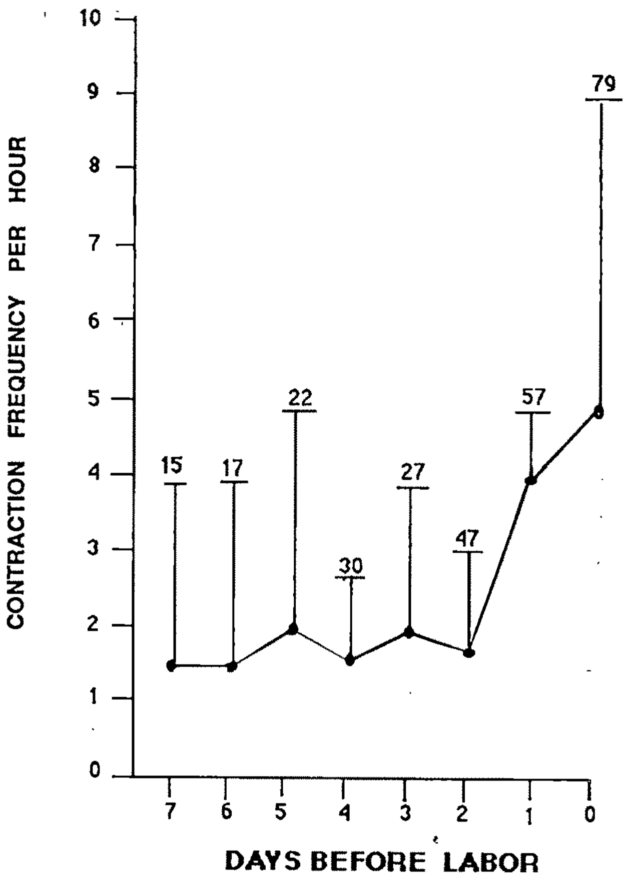

Fig. 1. Average number of contractions each day during expectant management in patients with preterm premature rupture of fetal membranes.

The major finding of this investigation is that most women with expectant management of preterm premature rupture of fetal membranes seem to have a pattern of uterine activity that is similar to that of women who later have preterm labor with intact membranes. Excessive rates of uterine contractility typical of impending preterm labor were not seen in the women immediately after preterm premature rupture of fetal membranes. It seems likely that excessive subclinical uterine activity is not a major factor in most cases of preterm premature rupture of fetal membranes. An obvious question concerns whether the uterine activity after preterm premature rupture of fetal membranes reflects uterine activity that may have preceded rupture of the membranes. It seems unlikely that excessive uterine activity before preterm premature

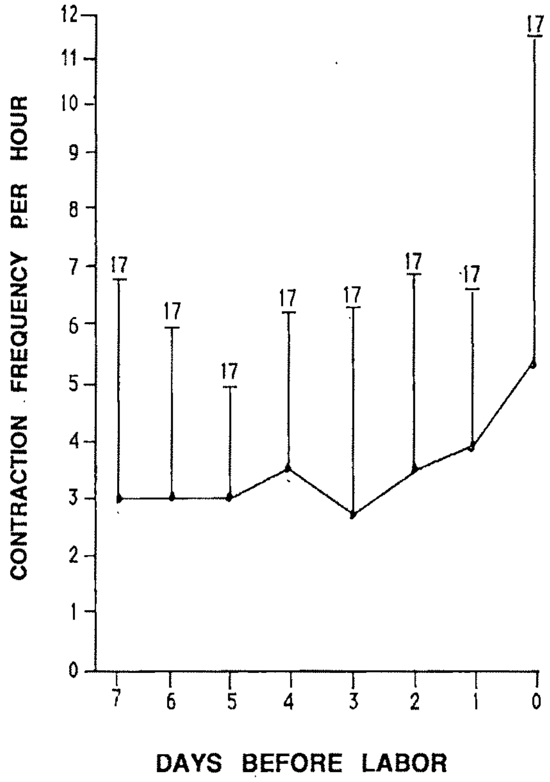

Fig. 2. Average number of contractions each day in patients with intact membranes and spontaneous labor. (From Katz M, Newman RB, Gill PJ. Assessment of uterine activity in ambulatory patients at high risk of preterm labor and delivery. Am J Obstet Gynecol 1986;154:44.)

rupture of fetal membranes would disappear after rupture. It is important to note that 35% of the women with preterm premature rupture of fetal membranes betweeen 26 and 34 weeks' gestation in our institution were not candidates for expectant management. A variety of reasons, including active labor, may explain why these women were not candidates for expectant management. Among these women, excessive uterine activity leading to preterm premature rupture of fetal membranes and then evolving to active labor easily could have occurred.

To optimally evaluate the relationship between uterine activity and preterm premature rupture of fetal membranes, it would be necessary to monitor uterine contraction frequency before rupture of the membranes. Clinical trials of home uterine activity monitoring have reported low rates of premature rupture of fetal membranes, which suggests that interventions to reduce excessive uterine activity may help prevent this complication.[8, 9] However, because of the difficulty in identifying women at risk for preterm premature rupture of fetal membranes and the ethical concerns raised by ignoring excessive prelabor uterine activity in women at such risk, it is unlikely that a definitive study of uterine activity preceding preterm premature rupture of fetal membranes will be possible.

During expectant management, the development of an excessive uterine contraction frequency (four or more contractions per hour) is a strong indicator of impending preterm labor. The onset of spontaneous preterm labor was preceded by an excessive contraction frequency in >70% of the patients studied. Martin et al.[10] previously reported very similar findings in a small group of patients with premature rupture of fetal membranes that was managed conservatively. Among women with preterm premature rupture of fetal membranes, the positive predictive value and sensitivity of a baseline contraction frequency of four or more contractions per hour were 54% and 72%, respectively. A contraction frequency of fewer than four contractions per hour was reassuring that labor would not ensue within the next 24 hours (negative predictive value, 95%, specificity, 90%). This predictive information regarding impending preterm labor could be helpful in the management of women with abnormal presentations who are at risk for cord prolapse or birth trauma. Some investigators have suggested the use of antibiotics and tocolysis to prolong pregnancy complicated by preterm premature rupture of fetal membranes.[11] The early identification of impending preterm labor may be of value in determining the role of ascending genital tract infection in premature rupture of fetal membranes and in maximizing the therapeutic potential of tocolytic agents. This information would be of great use if other management plans were used that placed a premium on the early diagnosis of preterm labor in women with preterm premature rupture of fetal membranes.

After preterm premature rupture of fetal membranes, the crescendo of uterine activity leading to the diagnosis of spontaneous labor is very similar to the pattern demonstrated by women with intact membranes. It appears that the status of the membranes does not affect the evolution of uterine activity necessary to initiate preterm labor. Although rupture of the membranes does not appear to be associated with excessive uterine contractility in most cases, the short latent period indicates that preterm premature rupture of fetal membranes is a powerful facilitator of preterm labor. We believe that the ultimate onset of labor in this patient population is often associated with an ascending decidual or chorioamniotic infection.

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Obstetric complications in a patient with Bernard-Soulier syndrome

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We describe the obstetric complications and management of a patient with Bernard-Soulier syndrome. Severe bleeding at the time of delivery and delayed postpartum hemorrhage were prominent features of her pregnancies. Further complicating this woman's pregnancies was the development of antibodies to platelet glycoprotein IB/IX, leading to neonatal alloimmune thrombocytopenia. (AM J OBSTET GYNECOL 1991;165:425-6.)

Key words: Neonatal alloimmune thrombocytopenia, Bernard-Soulier syndrome

Individuals may lack the membrane glycoprotein IB/IX, leading to Bernard-Soulier syndrome, which is characterized by platelet dysfunction. Life-threatening bleeding may occur spontaneously or after invasive procedures. Women lacking these glycoproteins are also at risk of being immunized by fetal platelets during pregnancy. We report on a mother with Bernard-Soulier syndrome who was immunized to platelet glycoprotein IB/IX, leading to neonatal thrombocytopenia.

Case report

A 35-year-old woman had Bernard-Soulier syndrome. Her first pregnancy, which occurred in 1972, was uneventful. Postpartum hemorrhage necessitated 11 units of blood. In 1986 her second pregnancy ended with in utero fetal death at 38 weeks' gestation, concomitant with severe third-trimester gastrointestinal bleeding. Vaginal bleeding necessitating 8 units of blood occurred 2 weeks after delivery. Treatment with aminocaproic acid significantly reduced the vaginal bleeding. In 1987 a third pregnancy resulted in an infant with a platelet count of 51,000/mm^3. In 1988 she was seen for her fourth pregnancy. At the onset of labor she received 50 gm γ-globulin intravenously. Vaginal delivery of a nonthrombocytopenic infant occurred 28 hours later. ε-Aminocaproic acid was initiated post partum, 5 gm intravenously, then 2 gm every 6 hours for 24 hours. Post partum she experienced moderate vaginal bleeding that necessitated four units of blood. After the addition of methergine, the bleeding decreased. The ε-aminocaproic acid was continued for 4 weeks.

Platelet antibody studies. After the third pregnancy the patient's serum was tested with an antiglobulin test that had a radioactive label against platelets from 20

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donors; it reacted strongly with all platelets. In the fourth pregnancy a sample collected during the first trimester showed similar serologic results. The serum was also tested in a solid-phase assay where antibodies to specific glycoproteins can be identified.[1] The antibody specificity recognized the platelet glycoprotein IB/IX.

Comment

The management of this case was complicated by abnormal platelets and alloimmunization to the fetus' platelets. We were reluctant to assess the fetal platelet count prenatally, because percutaneous umbilical blood sampling could lead to uncontrollable maternal bleeding. If bleeding should occur, we could not satisfactorily transfuse the patient with platelets because of alloimmunization to platelets. Second, for similar reasons cesarean section was not considered as an option for delivery. Third, in the event that fetal thrombocytopenia was diagnosed, suitable platelet transfusions were not available for in utero transfusion.

In our patient with Bernard-Soulier syndrome, through hundreds of platelet transfusions and multiple pregnancies, she became sensitized to platelet antigens. This was first suspected when she failed to receive any therapeutic benefit from platelet HLA-matched platelet transfusions. Subsequently we were able to document an antibody to platelet glycoprotein IB/IX. The unique obstetric problem of this patient related not only to the risk of maternal bleeding because of the qualitative platelet defect but also to the infant's risk of thrombocytopenia because of the passive transfer of platelet antibodies. Since the fetus of a mother with Bernard-Soulier syndrome would be heterozygous for the disorder, the fetal platelets carry antigenic determinants to which the mother's antibody can bind. We thought that this was the basis of the thrombocytopenia in the third pregnancy and that subsequent pregnancies would be complicated by this problem. In the fourth pregnancy these antibodies were again demonstrable.

Because of these considerations, we elected to treat the patient with intravenous γ-globulin in an attempt to reduce the risk of thrombocytopenia. In our patient management with intravenous γ-globulin appeared to be successful in preventing neonatal alloimmune thrombocytopenia. Unlike the pregnancy with her third child, the last pregnancy, in which intravenous γ-globulin was given, was not associated with a thrombocytopenic baby. Since we were unable to obtain pre–intravenous γ-globulin treatment fetal platelet counts, we cannot prove the efficacy of the intravenous γ-globulin.

Obstetric experience in women with Bernard-Soulier syndrome is not commonly reported.[2] Our experience suggests that women with Bernard-Soulier disease may be at risk for severe hemorrhage at the time of delivery. Because these patients may be alloimmunized to platelet glycoprotein IB/IX, platelet transfusions may be ineffective. Delayed bleeding up to 2 to 3 weeks post partum is not uncommon. Patients should be cautioned about this possibility. One should be cautious about performing any type of invasive procedure since uncontrollable hemorrhage may result. Our report also emphasizes that the obstetrician must consider the risk of neonatal alloimmune thrombocytopenia in the fetus and manage this problem accordingly.

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Acute cutaneous vasculitis associated with prolonged intravenous ritodrine hydrochloride therapy

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A patient with twin gestation was hospitalized because of preterm labor and treated with intravenous ritodrine hydrochloride (Yutopar, Astra Pharmaceutical Products, Westborough, Mass.). After >4 weeks of therapy, the patient had a petechial rash and prolonged bleeding time, which were diagnosed and confirmed by skin biopsy at cesarean section as vasculitis. This is the first documented case of vasculitis associated with ritodrine use. (AM J OBSTET GYNECOL 1991;165:427-8.)

Key words: Preterm labor, cutaneous vasculitis, ritodrine

Preterm delivery, before 37 weeks' gestation, occurs in 5% to 10% of all pregnancies in developed countries and accounts for 60% to 90% of perinatal mortality in infants without anomalies. β-Sympathomimetic amines are a well-accepted and frequently used treatment for preterm contractions since Food and Drug Administration approval in 1980. Ritodrine hydrochloride (Yutopar, Astra Pharmaceutical Products, Westborough, Mass.) is a β_2-adrenergic agonist that relaxes uterine muscle by a hypothesized cyclic adenosine 5′-monophosphate—mediated mechanism. Common side effects include tachycardia, nausea, hypokalemia, and flushing, which necessitate discontinuation of therapy in 3% to 10% of patients. More serious side effects include hypotension, cardiac arrhythmias, myocardial ischemia, and pulmonary edema, which have been observed in 1% to 5% of cases. In our institution we encountered a case of vasculitis, previously unreported, after prolonged β-sympathomimetic tocolysis.

Case report

A 30-year-old woman, gravida 1, para 0, was diagnosed by ultrasonography to have twins at 10 weeks' gestation. She had no significant medical or surgical history, including no history of arthritis, autoimmune disease, petechial rash, or thrombocytopenia. Platelet counts of 280,000/mm³ and 209.000/mm³ were measured at 18 and 14 months, respectively, before delivery. At 29 weeks' gestation, the patient noted mild contractions, and she was placed on bed rest and started on a regimen of oral ritodrine hydrochloride, 10 mg every 6 hours. One week later she felt increasing contractions and was admitted to the hospital in preterm labor. At that time she had a sonogram consistent with her dates, a cervical examination showing 2 cm dilatation and 50% effacement, and monitored contractions occurring every 4 to 6 minutes. Admission laboratory studies included white blood cell count, 6400/mm³; hematocrit, 35.6%; platelet count, 149,000/mm³ (150,000 to 450,000/mm³ is normal); fibrinogen, 380 mg/dl (200 to 400 mg/dl is normal); and antithrombin III, 91% (84% to 116% is normal).

She was begun on a regimen of intravenous ritodrine hydrochloride at 100 μg/min and contractions abated. Any attempt to change to a regimen of oral ritodrine or to decrease the amount of intravenous ritodrine resulted in return of contractions. On this medication regimen fibrinogen, hematocrit, and serum potassium level remained stable. She was continued on the regimen of intravenous ritodrine at 100 μg/min for 5 weeks. At 34 weeks' gestation (5 weeks after admission) a nonpalpable, nonpruritic petechial rash appeared initially on the forearms and later extended to all extremities. She had no mucosal bleeding, hematuria, hematochezia, or palpable hepatosplenomegaly. Hematologic studies included white blood cell count, 5000/mm³; hematocrit, 34%; platelets, 125,000/mm³; prothrombin time, 11.1 seconds (normal); partial thromboplastin time, 25.2 seconds (normal); fibrin degradation products, >10 <20 μg/ml; and template bleeding time, 13 minutes (normal, 2 to 9 minutes). Factors V (100%), VII (120%), and VIII (100%) and plasminogen (96%) were normal. Antithrombin III was decreased at 68%. Platelet aggregation was normal with the agonists adenosine 5′-diphosphate, collagen, ristocetin, epinephrine, and arachidonic acid. Other normal or negative studies included creatinine clearance (89 ml/min), antinuclear antibodies, rheumatoid factor, serum complement (C′3 and C′4), cryoglobulins, electrolytes, liver function tests, and fibrinogen (393 mg/dl). The findings of prolonged bleeding time and petechial rash, combined with normal platelet function and only slight thrombocytopenia, suggested vasculitis.

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Her only medications, other than ritodrine, were prenatal vitamins and mineral supplements.

Ritodrine was discontinued and 100 mg hydrocortisone was infused intravenously. Four hours later the bleeding time was normal (6.5 minutes), and the patient underwent an uncomplicated, low-segment, transverse cesarean section while she was under general anesthesia, with delivery of a 1700 gm male infant, with Apgar scores of 9 and 9 at 1 and 5 minutes, and a 2100 gm male infant, with Apgar scores of 3 and 9 at 1 and 5 minutes, respectively. The infants had normal platelet counts and no bleeding problems. Pathologic examination of a biopsy specimen of the abdominal skin at the incision site revealed a leukocytoclastic vasculitis, diagnosed by the presence of neutrophils and fibrin in the walls of small arterioles.

The patient received intravenous hydrocortisone (100 mg every 6 hours) for the first postpartum day. The rash improved with the corticosteroids, which were tapered and discontinued by the third day. She was discharged home on postoperative day 5 with minimal residual rash. Follow-up platelet count on postpartum day 7 was $224,000/mm^3$.

Comment

Ritodrine hydrochloride is a frequently used tocolytic for the commonly occurring complication of pregnancy, preterm labor. Our patient was seen with uncomplicated mild thrombocytopenia that was persistent throughout successful prolonged intravenous ritodrine therapy (5 weeks). A biopsy-proved acute cutaneous vasculitis developed with prolonged template bleeding time. Platelet count would not have been expected to result in a prolonged bleeding time, and in vitro platelet function tests were normal. She was otherwise healthy and not receiving any other medication, other than the ritodrine, that might be incriminated as a cause of either thrombocytopenia or vasculitis. We conclude that vasculitis was likely associated with ritodrine infusion. However, mild thrombocytopenia is less easily related to the drug, in view of the frequency of mild asymptomatic thrombocytopenia of unclear cause in late pregnancy.[1]

Only rarely have other hematologic and cutaneous complications been related to ritodrine. These include erythema multiforme[2] and neutropenia after 26 days of ritodrine.[3] We have been able to find no other reported cases of vasculitis or thrombocytopenia associated with ritodrine use, and the manufacturer also is unaware of any. Discontinuation of ritodrine and administration of corticosteroids were followed within 4 hours by normalization of the bleeding time, which permitted uncomplicated twin delivery by cesarean section. The rash subsided over several days, and the platelet count returned to antepartum levels by the seventh postpartum day. This report should alert the clinician to the possibility of unusual hematologic and cutaneous complications associated with prolonged parenteral ritodrine therapy.

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The three most common occupational exposures reported by pregnant women: An update

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Many uncertainties exist in regard to counseling women with occupational exposures during pregnancy. This is due to limited knowledge of the reproductive toxicologic effects of industrial agents, lack of safety standards aimed at protecting the fetus, and limitations in assessing the extent of exposure. The approach to this subject taken by the Motherisk Program and a review of the three most common occupational exposures are presented. Epidemiologic studies and measurements of radiation do not suggest a reproductive hazard for video display terminals. Exposure to organic solvents is hard to quantitate, and information is sparse and sometimes contradictory, and therapeutic decisions are difficult to reach. To date there is no convincing evidence that working with organic solvents within safety regulations would harm a fetus, in contradistinction to detrimental fetal effects of solvent abuse. The reproductive risks of lead are well documented, fetal exposure can be assessed, and effective treatment exists, but its effects on the pregnancy have not been fully established. However, new evidence suggests that maternal levels that are much lower than the accepted adult limits may be damaging to the fetus. (Am J Obstet Gynecol 1991;165:429-37.)

Key words: Video display terminals, organic solvents, lead, pregnancy, fetus

It is clear from animal experiments and human epidemiologic studies that industrial chemicals have the potential of being reproductive toxins. However, the knowledge of reproductive toxicology of industrial chemicals in humans is sparse or absent. Before therapeutic agents are marketed, their teratogenic potential has to be tested in animals; no such data have to be presented for chemicals. Other factors may also be different between medical and occupational exposures. In the workplace the exposure is usually to more than one chemical, and in some cases one has to deal with possible unknown by-products. The amounts of the chemicals absorbed are often unclear, and the circumstances of exposure may vary between different plants or even within the same operation. Every chemical in the workplace has safety exposure limits aiming at protecting the worker. However, these standards were not meant to protect the fetus. Hence, even if one can obtain exposure levels, one is never sure whether safe levels to the mother are also safe for her unborn baby; lead is a good example of such a discrepancy.[1, 2]

To approach the occupationally exposed woman the following steps may be suggested:

1. Identify the chemicals in question, by their safety sheets if possible.
2. Identify symptoms and signs reported to be associated with the chemicals.
3. Rule out underlying conditions that may cause a similar clinical picture (e.g., morning sickness).
4. Obtain a detailed description of the work performed by the woman, length of exposure, means of protection (ventilation system, respirator, mask, gown, gloves, hood, etc.).
5. Are there symptoms and signs in fellow workers?
6. What is the pregnancy outcome in other workers?
7. Obtain the most recent levels of the chemicals in question measured in that particular area.
8. Try to understand the attitude of the woman and her supervisors toward her particular work and toward possible change of job; will it affect her income or promotion?
9. Before reporting to the woman on available information, evaluate the data critically to allow the patient accurate information.
10. Advise the woman on possible safety measurements to reduce exposure (mask, gloves, ventilation, etc.).

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6/1/29567

Table I. Distribution of telephone consultations for occupational exposures

No.	*Video display terminal*	*Organic solvents*	*Lead*	*Miscellaneous*
5040	167	56	6	13

All these steps should be completed by obtaining a good medical history, estimating the gestational age, evaluating genetic diseases, and assessing the presence of other risk factors (alcohol use, cigarette smoking, concomitant drugs, chronic diseases, etc.).

The Motherisk program in Toronto is an antenatal counseling service for health professionals, women and their families, dealing with drug, chemical, radiation, and infection exposure in pregnancy and lactation. Presently, between 40 and 50 telephone calls are dealt with daily. Women are referred to our clinic if they have been exposed to known or suspected teratogens, long-term drug therapy, new drugs on which there is sparse or no information, drugs of abuse, and occupational exposures. After the expected date of confinement, follow-up of pregnancy outcome is performed, and all data are computerized for clinical and research use.

In the Motherisk Program we are frequently called about exposure to chemicals and radiation during pregnancy. During 1988 we received 5040 telephone calls; 167 (3.3%) of them were due to occupational exposures to video display terminals (Table I). This exposure is the most frequent one that we encounter in the workplace. Organic solvents were the concern of up to 150 of the telephone calls, most of them due to the use of oil-based paints, some of them at home. In 56 the exposure to organic solvents occurred in the workplace, and 24 of these were followed up in our clinic (Table II). We also advised 29 patients who were exposed to lead (most of them in the form of paints); six were occupationally exposed, and three were seen in the clinic.

The purpose of this article is to review the state of our knowledge on the reproductive hazards of these three exposures, to give the clinician up-to-date information with which to advise women.

Video display terminals

Over the last years the microprocessor has brought video display terminals into offices and homes. and a massive increase in their use has been observed. It was estimated that in the United States 7 million people were occupationally exposed to video display terminals in 1980.[3] The expansion of their use has prompted concerns about their reproductive effects.

About 10 years ago clusters of cases of adverse pregnancy outcome in workers exposed to video display terminals were reported, some of them in the lay media.[4-7] The most widely publicized cluster was reported by the *Toronto Star*, where over a year's time four of seven pregnant women working with video display terminals gave birth to children with birth defects.[6] These defects were different in each affected child and included clubfoot, underdeveloped eye, cleft palate, and a congenital heart defect. Additional clusters involved spontaneous abortions, Down syndrome, neural tube defects, prematurity, and neonatal respiratory disease. Evidently, none of these reports represents a distinct or reproducible syndrome. Miscarriages, for instance, are not evenly spread in time and space and some clustering is to be expected. Hence the occurrence of clusters does not prove causality but rather may increase the level of suspicion. A partial answer may come from looking at the physical properties of video display terminals and from epidemiologic studies.

The ionizing radiation generated by the video display terminal consists of x-rays, the adverse reproductive effects of which are well known. However, x-rays emitted by the cathode ray tube in the video display terminal are entirely absorbed by the glass screen.[8] Several studies could not detect measurable ionizing radiation from video display terminals (0.01 to 0.05 mR/hr.[8-12] This was also the case in the reported clusters where the video display terminals involved were tested. Current regulations require that x-ray emissions from any cathode ray tube, including video display terminal, will be <0.5 mR/hr.[4] Even at this maximum level the amount of uterine radiation for a woman sitting 50 cm from the screen, working 30 hours a week, is 6 mrem during the first trimester.[13] This is one fourth of the natural background radiation, which is by itself very minimal.

The nonionizing radiation transmits energy to tissues mainly as heat and it includes the following:

1. Ultraviolet and visible light (emitted by the phosphor target of the screen) are two to five orders of magnitude less than that of the environment.[9, 11] The amount of heat they produce is very small ($1.72 \cdot 10^{-6}$ cal) and their wavelength is not in the dangerous range (>336 nm; harmful, <300 nm).[11]
2. Infrared radiation has never been detected from the video display terminals tested.[9, 11]
3. Microwaves and radiofrequency radiation emitted are in the same frequency range as that given off by most home electrical appliances.[9] This amount of radiation is two orders of magnitude less than the background.[11] Some video display terminals are equipped with a flyback transformer, which is responsible for moving the arm of the cathode ray tube back and forth. People coming near the back casing of such a unit may be exposed to a lower-

frequency electromagnetic radiation, which is thought to be more dangerous.[6] This problem was solved with modified casing.

In short, ionizing radiation is practically unmeasurable and the amount of nonionizing radiation is very small, several orders of magnitude less than the background radiation, and cannot be considered as harmful to the fetus.

Epidemiologic studies might be fraught with pitfalls. Confounding factors include reproductive history (miscarriage, anomalies), maternal age, maternal smoking, selection bias, and recall or response bias between the exposed and the control groups. So far, no epidemiologic study has demonstrated a higher risk of miscarriages and birth defects.[14-18] Some studies that initially suggested some association were found by the authors themselves to be subjected to selection bias and recall bias,[14, 18] and in another one response bias seemed to be a possible explanation for such an association.[19]

On the basis of current data and because a very large number of women are exposed occupationally to video display terminals without emergence of a peak of any specific pattern, it seems likely that the clusters reported were encountered by chance.[6]

Consequently, in the Motherisk Program we do not feel that there is a need for a concern or a change in working conditions. We may suggest, in very selected cases, as is done in other countries (Sweden, England), that women may request to leave work with video display terminals on the grounds of reducing anxiety only.[20, 21]

Organic solvents

In our clinic we often counsel women who are exposed occupationally to numerous chemicals, most of which are organic solvents. A proper consultation in such cases is extremely difficult, because it is hard to estimate the predominant chemicals and their byproducts. Even if one identifies the more toxic ones, it is still hard to assess the circumstances of exposure. For many chemicals one can measure neither airborne nor blood levels. Smelling the odor of organic solvents is not indicative of a significant exposure, because the olfactory nerve can detect levels as low as several parts per million, which are not necessarily associated with toxicity. Finally, reproductive information on most solvents is sparse, either limited to animal studies or nonexistent.

Organic solvents are a structurally diverse group of low-molecular-weight chemicals that are liquids and are able to dissolve other organic substances.[22] They are ubiquitous in our industrialized society, both at work and at home, and may be encountered as individual agents or in complex mixtures such as gasoline. Chemicals in the solvent class include aliphatic hydrocarbons (such as mineral spirits, varnish, and kerosene), aromatic hydrocarbons (benzene, toluene, xylene), halogenated hydrocarbons (carbon tetrachloride, trichloroethylene, methylcellulose), aliphatic alcohols (acetone), glycols (ethylene glycol), and glycol ethers (methoxyethanol).[23, 24] Fuels are mixtures of various hydrocarbons.

Table II. Distribution of occupational exposures with clinic follow-up in 1988

	No.	*Total clinic visits* (%)*	*Occupational exposures seen in clinic† (%)*
Organic solvents‡	24	6.3	66.6
Video display terminals§	5	1.3	13.8
Lead‖	3	0.8	8.3
Polychlorinated biphenyl	2	0.5	5.5
Miscellaneous¶	5	1.3	13.8

*A total of 380 patients were seen in the clinic.

†Thirty-six patients with occupational exposures were monitored in the clinic.

‡Two patients were exposed to organic solvents and lead.

§Four patients came to the clinic because of drug exposure.

‖Two patients were exposed to lead and organic solvents.

¶Each patient was exposed to multiple chemicals, no organic solvents.

The mechanisms by which many solvents exert their toxicity are unclear and may vary from one solvent to another. Halogenated hydrocarbons such as carbon tetrachloride may generate free radicals.[22] Simple aromatic compounds such as benzene may disrupt polyribosomes,[25] whereas some solvents are thought to affect lipid membranes and penetrate tissues such as the brain.

Incidental exposures may include vapors from gasoline, lighter fluid, spot removers, aerosol sprays, and paints.[26] These are short-duration, low-level exposures and may go undetected. More serious exposures occur mainly in industrial or laboratory setting during manufacturing and processing operations. This includes paint removers, thinners, floor and tile cleaners, glues, and laboratory reagents. Gasoline or glue sniffing, although not occurring in the occupational setting, are other sources of exposure to organic solvents during pregnancy.

Workers with short-term exposure to organic solvents experience fatigue, concentration disorder, drunken feeling, dizziness, pneumonitis, and vomiting.[21] Long-term exposure may irreversibly affect the central nervous system and liver and may cause blood disorders (e.g., exposure to benzene).

Although the toxic effects of organic solvents are relatively known in the adult, there is a paucity of information on the impact of in utero exposure.

In 1988 our patient population included 150 (2.9%) cases of telephone counseling and 24 clinic consultations dealing with organic solvents (Tables I and II). Most of the occupationally exposed women were seen in the clinic, and most of them were involved in manufacturing, processing, and application of paints and glues. Others were machinists, laboratory technicians, and dry cleaners.

It is beyond the scope of this article to review all the organic solvents, so we chose toluene as an illustrative example of exposure. This agent is used in a variety of mixtures and products. Also important, it is a common substance of abuse. Toluene is an aromatic hydrocarbon used as a solvent for paints, thinners, coatings, and glues.[23] It is a popular replacement for the more chronically toxic benzene solvents. Most exposures involve inhalation; however, absorption is almost complete after oral administration.[23] Toluene inhalation by pregnant rats induced decreased fetal weight and skeletal growth retardation.[27] No malformations were demonstrated in rats or mice after inhalation,[27, 28] but oral administration to mice induced cleft palate.[29] A study from 1977 compared pregnancy outcomes among 168 women occupationally exposed to varnishes containing toluene (55 ppm) with those of 201 control women.[30] While there were twice as many low-birth-weight infants in the toluene-exposed group, the two groups did not differ with regard to fertility, course of pregnancy, perinatal mortality, or adverse effects in the newborn. Unfortunately, congenital defects were not evaluated. Occupational exposure to aromatic solvents, mainly to toluene, was suggested to be associated with various birth defects, predominantly renal-urinary or gastrointestinal.[31] Abuse of large quantities of pure toluene by inhalation throughout pregnancy was reported in three patients to result in microcephaly, central nervous system dysfunction, and minor craniofacial and variable growth deficiencies.[32] These features resembled the pattern of malformations described with alcohol or certain anticonvulsant medication exposure and were defined as "fetal solvent syndrome."

Many organic solvents are teratogenic and embryotoxic in laboratory animals, depending on specific solvent, route of administration, and animal species.[22, 26] Malformations described include hydrocephalus, exencephaly, skeletal defects, cardiovascular abnormalities, and blood changes. Other abnormalities include poor fetal development and neurodevelopmental deficits. In some of the studies exposure levels were high enough to induce maternal toxicity.

Because of the complexity and diversity of organic solvents and because exposure usually involves more than one agent and different circumstances, adequate human epidemiologic studies are difficult to conduct and interpret. In addition, studies are subjected to recall and response bias and are not always controlled for other risk factors (age, smoking, etc.). Isolated case reports suggesting solvent-related embryopathy to occur in humans have appeared for many years. In one report five of nine women who gave birth to infants with caudal regression syndrome were exposed to solvents, including xylene, trichloroethylene, methyl chloride, acetone, and gasoline.[33] These agents do not belong to the same subgroup of organic solvents, and it is impossible to identify the potential culprit. Several epidemiologic studies suggested association between adverse pregnancy outcome and exposure to organic solvents. Although these solvents may have common chemical features, there is no indication that they were teratogenic as a group or on an individual basis. These studies reported esophageal stenosis or atresia in babies of female laboratory workers,[34] omphalocele or gastroschisis in offspring of mothers in the printing industry,[35] an association between increased risk of malformations and laboratory work in the pharmaceutical and paper industries.[36, 37] Case-control studies of central nervous system defects in Finland showed an association with organic solvents.[38, 39] However, another study from Finland could not prove this association.[40] A cumulative case-referent study covering 3.5 years suggested an association of organic solvent with cleft palate.[41] The prevalence of exposure to organic solvents at work during the first trimester was 10.4% among 569 mothers of children with cardiovascular malformation compared with 7.8% in the control group.[42] This retrospective study found an adjusted relative odds ratio of 1.3 for cardiovascular malformations and 1.5 for ventricular septal defects, both probably insignificant. A recent study from California could not demonstrate any difference in neurobehavioral development and growth between children exposed in utero to organic solvents and a group of matched, unexposed children.[43] A recent study published by the same group suggested an association between exposure to organic solvents and preeclampsia.[44] No correlation was found between laboratory work and sister chromatid exchanges and micronuclei in 59 Canadian laboratory workers.[45] There was, however, an association between such exchange and recent or past smoking.

A higher risk for spontaneous abortions was reported in hospital laboratory and factory workers.[46, 47] This was also the case in 262 factory workers in Denmark.[48] However, when controlled for gravidity there was no longer a statistically significant increased risk. No increased risk for miscarriage was found in university laboratory workers exposed to organic solvents.[49] Conversely, shift work in these workers was related to a higher miscarriage rate (relative risk 3.2). This study did not demonstrate any differences in perinatal death rates or in the prevalence of malformations among women working with organic solvents when compared with controls. In a recent case-referent study, the odds ratio of spontaneous abortions was increased by paternal exposure to several organic solvents, maternal exposure to organic solvents, and maternal heavy lifting.[50] The cohort was too small to evaluate the effect of these parameters or congenital malformations.

Discussing organic solvents will be incomplete without mentioning the fetal solvent (or gasoline) syndrome. In 1979 a syndrome of anomalies (hypertonia, scaphocephaly, mental retardation, and other central nervous system effects) was suggested in two children in a small American Indian community where gasoline sniffing and alcohol abuse are common.[51] Four other children had similar abnormalities, but in their cases it was impossible to verify gasoline sniffing. It is unclear what was the contribution of the lead in the gasoline or the alcohol abuse in producing these abnormalities. In another case a child with nearly classic fetal alcohol syndrome was born to a mother with major addiction to solvents, mainly toluene.[52] Heavy alcohol consumption also was reported in that woman, and the authors questioned a possible interaction between solvents and alcohol. Toluene embryopathy was described in two additional children whose mothers probably did not abuse alcohol.[53] Paint sniffing, namely toluene, resulted in severe renal tubular acidosis in five pregnant women.[54] Fetal heart rate tracing and dynamic ultrasonographic examinations were normal in four of five. Three neonates exhibited growth retardation, and two had anomalies and hyperchloremic acidosis. It is important to remember that the mothers in all these cases showed signs of solvent toxicity, indicating heavy exposure. In our experience this is not the case in most occupational exposures during pregnancy.

In summary, it appears that organic solvents may have the potential to induce congenital defects and other pregnancy complications. However, it is hard to prove or quantitate it, certainly not for solvents as a group. While fetal toxicity is biologically sensible in cases of intoxicated mothers, the evidence of fetal damage from levels that are not toxic to the mother is scanty, inconsistent, or missing.

Lead

The third most common occupational exposure in pregnancy encountered by us is lead. It accounted for 29 (0.6%) of the telephone consultations provided by the Motherisk Program during 1988. Three of them were followed up in our clinic (Tables I and II). The vast majority of lead exposures consisted of artists using glass staining or workers involved in paint manufacturing in the automotive and aircraft industries. Other occupational sources of lead exposure during pregnancy reported in the literature include the printing, smeltering, and battery industries.[55] Not only is lead an occupational hazard, but also it can enter the body from contaminated soil and drinking water,[56] by residence close to industrial areas or from a lead-exposed spouse,[57] and by consumption of moonshine whisky.[58]

Although it seems that blood lead levels in the United States are declining,[59] levels are now being considered toxic at a much lower range than in the past (<25 μg/dl in children, <50 μg/dl in adults).[60] Lead intoxication is still a hazard in the industrialized countries, including in the pregnant woman.

Lead crosses the placenta,[61, 62] possibly by both passive diffusion and active transport,[60] and it is unclear whether placental permeability to lead is constant throughout gestation.[61, 63-65] Transplacental transfer of lead has been shown in the human fetus as early as 12 to 14 weeks of gestation, along with increasing amounts of lead in fetal tissues with advancing gestational age.[66] Fetal bone and liver may have higher lead concentrations than maternal tissues.[67] Pregnancy, in a sense, is a stressor that may mobilize lead from maternal skeletal storage sites.[55] Iron and calcium deficiencies, both of which may exist in pregnancy, further increase the susceptibility to lead toxicity.

During the late nineteenth and early twentieth centuries women in the pottery and white lead industries used lead as an abortifacient.[55] European studies from that time found infertility, abortion, stillbirth, fetal death, and microcephaly to be associated with industrial lead exposure.[57, 68-71] Even paternal lead exposure was found as early as 1860 to affect fertility and viability of the offspring.[72] Wives of lead workers were reported to have more abortions, stillbirths, and premature births than in the general population.[57, 73] Consequently, the employment of women in plants involving a lead hazard was forbidden.[74] Although these studies should be evaluated with consideration of the high fetal and neonatal loss for other working women at that time,[75] most of the studies from the 1950s and later confirmed the early observations. For example, a study comparing the course and lead values in 249 pregnancies in Columbia, Mo., with 253 occurring at the center of America's lead belt at Rolla, Mo., showed that 96% versus only 70% were delivered at term.[76] In addition, 17% of the lead-exposed pregnancies had premature rupture of membranes, as compared with only 1% in the nonexposed group. Maternal and fetal blood lead levels in the cases of premature membrane rupture and preterm delivery were higher than in controls. A detailed Japanese study showed an increase in spontaneous abortion among female lead workers from a prelead rate of 45.6 per 1000 to 84.2 per 1000 (the rate in nonexposed employees was 59.1 per 1000).[77] A Danish study showed that when lead was used as an abortifacient, 60% of the pregnancies in the first trimester ended in abortion.[78]

A recent study from Boston (4354 cases) suggested lead to be associated, in a dose-related fashion, with an increased risk for minor anomalies.[79] The relative risk increased from 1.0 at 0.7 μg/dl to 2.73 at the level of 24 μg/dl. The anomalies discovered did not have a specific pattern and were of little health consequence. They included hemangiomas (14 of 1000 births), hydrocele (27.6 of 1000 male infants), minor skin anomalies (12.2 of 1000 births), and undescended testicles (11 of 1000 male infants).

Lead also affects the male gonads; chromosomal alterations, and abnormalities in sperm count, vigor, and morphologic features were demonstrated in workers and experimental animals.[80-82] Marital life records of lead-exposed men showed 24.7% of the marriages to be infertile compared with 14.8% in the nonlead control group. The rate of prematurity or stillbirth was 8.2%, whereas in the control group it was 0.2%.[82]

Although increased numbers of chromosomal aberrations (gaps, breaks, fragments, chromatid aberrations) have been reported in lead workers, it is still unclear what is the contribution of lead itself.[55]

Lead also seemed to have a small but demonstrable association with pregnancy-induced hypertension and elevated blood pressure at the time of delivery, but not with preeclampsia.[83, 84]

One of the main concerns regarding lead is its ability to cause neuropsychologic impairment in children. In the late 1970s 40% of children in the United States under 5 years of age had blood lead levels >20 μg/dl, and among urban black children the figure approached 60%.[85] At this level a variety of enzymatic and neurophysiologic processes are impaired. Until recently, it was unclear at what level deficits in children's learning and behavior become apparent and what is the contribution of in utero exposure. In a series of studies from Boston,[1, 2] blood lead levels and development were monitored in a group of urban children from birth to the age of 2 years. No infant had a cord blood lead level >25 μg/dl, the level that is regarded by the Centers for Disease Control as the upper normal limit for children. At all ages children whose cord level was >10 μg/dl scored lower in the Mental Development Index of the Bayley Scales (4.8 point difference between the low [<3 μg/dl] and high [≥10 μg/dl] groups). At the age of 6 months scores on the Psychomotor Development Index were not significantly related to cord blood lead level. Scores were not related to infants' postnatal blood lead levels. In a study from Cincinnati similar results were obtained.[86] This relationship between low-level lead exposure and neurobehavioral development was not confirmed in another study.[87] However, the mean cord lead level was 8.1 μg/dl, which is lower than the cutoff point of 10 μg/dl suggested by the Boston study. It is still unclear at what gestational age the fetus is most sensitive. However, because the fetal brain develops throughout gestation, it is conceivable that lead deposition at any stage may be detrimental.

These data suggest that low levels of lead delivered to the fetus may be toxic and may cause behavioral and developmental impairment. These levels are lower than the level defined by the Centers for Disease Control as acceptable for young children (25 μg/dl). Animal studies are consistent with these findings,[88-92] and it is possible that brain lead levels remain elevated longer than blood levels after short-term exposure.[93, 94] It is also possible that the fetus is more sensitive to lead than the young child. Possible mechanisms for the brain damage include interference with embryonic nutrition and energy supply, competition with cations such as zinc, iron, or calcium,[95] interference with mitochondrial function,[96] and synthesis of cytochromes, and effect on deoxyribonucleic acid synthesis.[97]

In a recent study we found that in Toronto about 90% of newborns had cord blood lead levels <3 μg/dl and none >10 μg/dl,[98] whereas in Boston about one third had such levels and one third had levels >10 μg/dl. This illustrates the difference between different urban environments.

The above data imply that it is important to detect, as early as possible, babies potentially exposed in utero to excess lead. Several studies documented a high correlation between maternal and cord blood lead levels.[61, 64, 99-101] In one of them mean cord levels were 10.1 μg/dl, compared with 10.3 μg/dl in the mother ($r = 0.6377$).[64] However, it is not advised to assume lead exposure throughout pregnancy from a single blood measurement, because of the changes in maternal blood levels and placental permeability to lead.[2] An alternative method to assess long-term lead exposure and estimate in utero exposure is head hair analysis, which reflects cumulative values.[102, 103]

On the basis of the higher susceptibility of the fetal brain to lead it may be argued that treatment should be instituted even when levels are lower than those accepted now as normal; however, the effectiveness of this approach has not been studied. More realistically, removal of the woman from the source of exposure is the first step. From the chelating agents available, BAL (dimercaprol) is a very toxic compound that has been shown to induce skeletal abnormalities in fetal mice.[104] In one pregnant woman with arsenic intoxication, after treatment in the sixth month of pregnancy a normal child was born.[105] Ethylenediaminetetraacetate is less toxic but it can chelate calcium, zinc, and other trace elements and may adversely affect the fetus. In one case it was given at the eighth month of gestation (at maternal level of 240 μg/dl) without complication.[75] The infant was normal with an undetected cord lead level. However, the sensitivity of the assay was very low (<60 μg/dl was undetectable). This infant had normal mental and developmental assessments at age 4. In another patient treated with calcium ethylenediaminetetraacetate at the same gestational age, it appeared that the treatment did not adequately reduce the infant's lead burden[106]; this child had a blood lead level of 60 μg/dl at the time of delivery and radiologic evidence of bony changes suggestive of prolonged exposure. D-Penicillamine is less effective than dimercaprol and calcium ethylenediaminetetraacetate, and in the doses used in lead poisoning it may cause connective tissue abnormalities (mainly cutis laxa) in human fetuses.[107] There is no experience with the use of the new oral chelators 2,3-dimercapto-succinic acid and dimercaptopropane-1-sulfonate in pregnancy. Since these chelators are less toxic, it is hoped that they will provide the answer to the question of the appropriate chelators in pregnancy. More critical research is needed to assess the need for treatment of low-level exposures, the criteria for instituting therapy and for assessment of response and the chelator of choice.

In the case of a woman occupationally exposed to lead, it is important to measure her blood lead concentrations and to compare them with the mean levels measured in the same city. In our practice several women working in glass staining had lead levels well below or near the mean recently determined by us for women in Toronto,[98] indicating that their lead load was within the expected. Should there be a substantially higher lead level in such patients, they would be advised to discontinue their occupational exposure.

In summary, of the three most common occupational chemical exposures in pregnancy, video display terminals do not represent a reproductive risk. Organic solvent may damage the fetal brain at high exposure levels, such as those encountered in substance abuse. There is no clear evidence to suggest that maternal exposure to allowable levels causes fetal damage. In the case of lead a dose-response fetal risk appears to have been established, and lead levels should be monitored to avoid fetal risk.

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Effects of vibration frequency and tissue thickness on intrauterine sound levels in sheep

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Pure-tone sinusoidal mechanical vibratory stimuli ranging in frequency from 4 to 4000 Hz were applied to the ventral abdominal wall of pregnant ewes, and intrauterine sound pressure levels were recorded with a hydrophone 5, 10, 15, and 20 cm from the surface. There were significant decreases in sound pressure levels with increasing frequencies ($p < 0.0001$) and increasing depth ($p = 0.01$). There was no significant interaction between these two variables. An electronic artificial larynx was also activated on the abdominal wall, resulting in quite high sound pressure levels (mean, 119 dB) measured 5 cm from the surface, with a significant decrease in sound pressure levels as the thickness of the intervening maternal tissues increased ($p = 0.005$). These results suggest that the frequency of vibratory stimulus used and the thickness of the maternal abdominal wall could influence fetal response to the vibroacoustic stimulation test by affecting intrauterine sound pressure levels. (AM J OBSTET GYNECOL 1991;165:438-42.)

Key words: Fetal sound, antepartum testing, acoustic stimulation, sheep

The vibroacoustic stimulation test has become popular as a test of fetal well-being. The factors that affect intrauterine sound pressure produced by the vibratory devices used for this test have not been adequately addressed. An understanding of these factors is important to develop testing procedures in which fetuses receive reproducible stimuli of appropriate intensity.

In an earlier study, Gerhardt et al.[1] reported high sound pressure levels produced by an electronic artificial larynx applied to the abdomen of pregnant ewes and recorded with a hydrophone sutured to the fetus. The sound pressure levels directly under the vibrator averaged 135 dB and decreased to 101 dB as the vibrator was moved 6 inches lateral from this point. In this study we concerned ourselves with two factors which could be expected to alter intrauterine sound levels in the vibroacoustic stimulation test: (1) the depth between the vibratory source applied to the surface of the abdomen of ewes and a hydrophone located inside the uterus and (2) the frequency of the stimulus.

Methods

Four near-term pregnant sheep were studied. Guidelines approved by the University of Florida for the care and use of animals were followed. The animals had been used for other experiments, then killed with an overdose of thiopental sodium and potassium chloride. Each animal was placed on its side, and the fetus was removed through a flank incision and hysterotomy. A miniature hydrophone (model 8103, Bruel & Kjaer, Marlborough, Mass.) supported on the end of a rigid Plexiglas acrylic rod was placed through the flank incision into the uterus, and the uterus was refilled with saline solution to remove air. The incision in the flank was made large enough so that the hydrophone could be moved in a direct line from 5 to 20 cm away from the ventral surface of the abdominal wall while maintaining its intrauterine position. The rod holding the hydrophone was held perpendicular to the table (Fig. 1).

Vibration excitation was produced by a shaker (model PM 50, MB Electronics, New Haven, Conn.) matched with a power amplifier (model 2250, MB Electronics). The shaker actuator rod was drilled and tapped to accommodate a circular stainless table (area, 1.32 cm^2) that was pressed against the ventral midline of the ewe with enough force to indent the skin by 1 cm. Sine waves were produced by a frequency generator (model 3010, Synascon Corporation, Chicago). Voltages driving the shaker were held constant at 2.5

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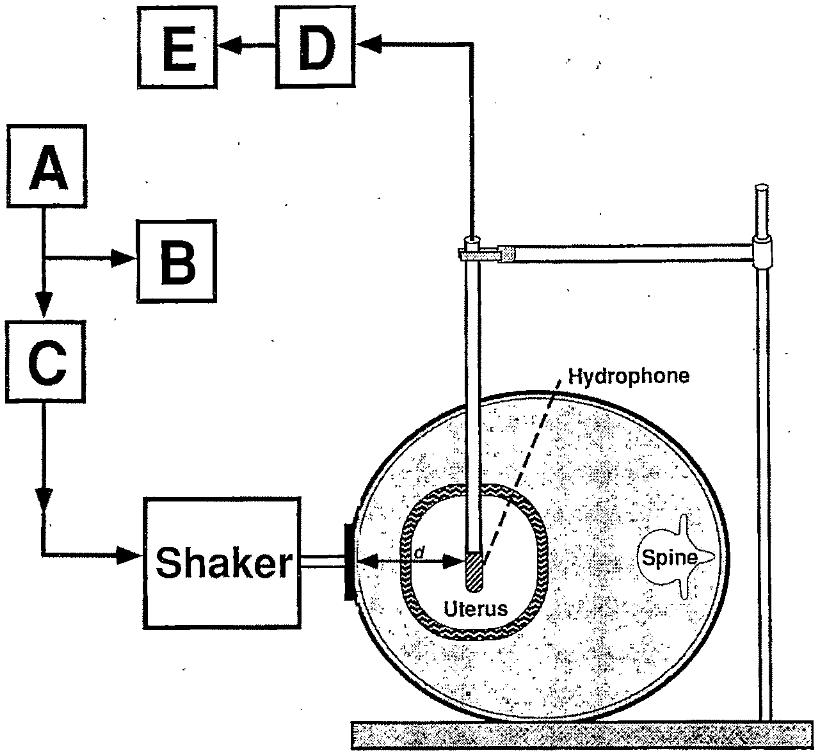

Fig. 1. Schematic drawing showing position of hydrophone within uterus and arrangement of devices for generating vibration and measuring sound pressure levels. *A,* Sine wave generator; *B,* frequency counter; *C,* power amplifier; *D,* charge amplifier; *E,* frequency analyzer; *d,* distance of hydrophone from shaker piston.

V between 4 and 500 Hz and at 7.5 V between 750 and 4000 Hz.

For each animal vibration at 17 different frequencies, ranging from 4 to 4000 Hz, was generated, first with the hydrophone 5 cm from the skin surface, then repeated with the hydrophone at depths of 10, 15, and 20 cm from the vibration exciter. At each depth, an electronic artificial larynx (model 5C, AT&T Consumer Products, Parsippany, N.J.) was also placed against the ventral abdominal wall overlying the hydrophone and activated.

The output from the hydrophone was amplified and conditioned with a charge amplifier (model 2635, Brull & Kjaer) and fed into a real-time frequency analyzer (model 2123, Brull & Kjaer). For pure-tone vibration, at each frequency and distance the sound pressure level (20 μPa) in 1/24 octave bands were measured at the fundamental frequency and first harmonic. The overall sound pressure level was recorded on linear scale, which is a measure of the sound pressure across the entire spectrum. Sound pressure levels were corrected for the higher voltages used to drive the vibration exciter at the higher frequencies. In the analysis of the levels produced by the electronic artificial larynx, only the linear value was used, because this device produces vibration across a wide range of frequencies.

The effects of vibration frequency and hydrophone depth on sound pressure levels were analyzed by repeated measures analysis. The effects of hydrophone depth on levels produced by the electronic artificial larynx were studied independently, with repeated measures analysis.

Results

For the electronic artificial larynx there was a statistically significant decrease in sound pressure level as the depth of the hydrophone increased from 5 to 20 cm (Fig. 2) ($p = 0.005$). The average fall in sound pressure level was from 119 dB at 5 cm depth to 102 dB at 20 cm.

With pure-tone vibration, analyzed at the fundamental frequency, there was a marked inverse relationship between vibration frequency and the resultant sound pressure level ($p < 0.0001$) (Fig. 3). The mean sound pressure level at a depth of 5 cm fell from 144 dB at 4 Hz to 101 dB at 4000 Hz. A similar fall was observed at all other depths. There was a less marked, but statistically significant, inverse relationship between

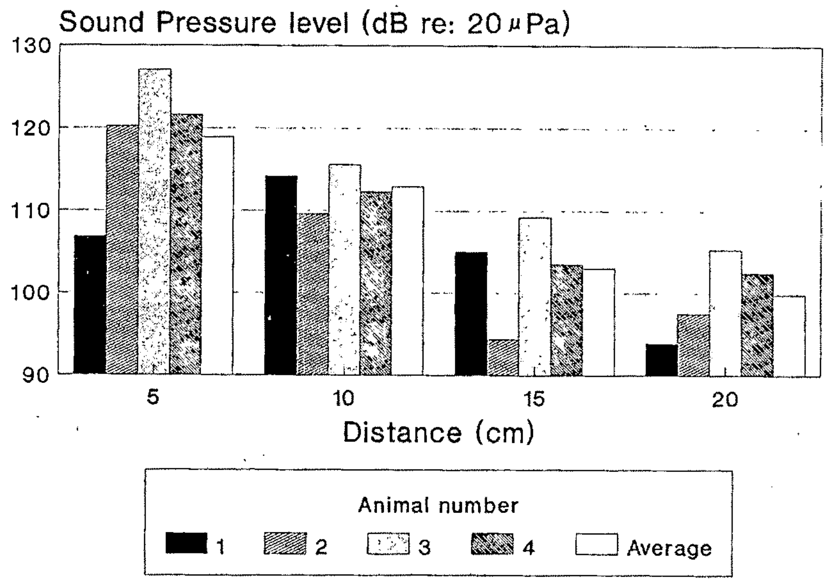

Fig. 2. Intrauterine sound pressure levels produced by electronic artificial larynx.

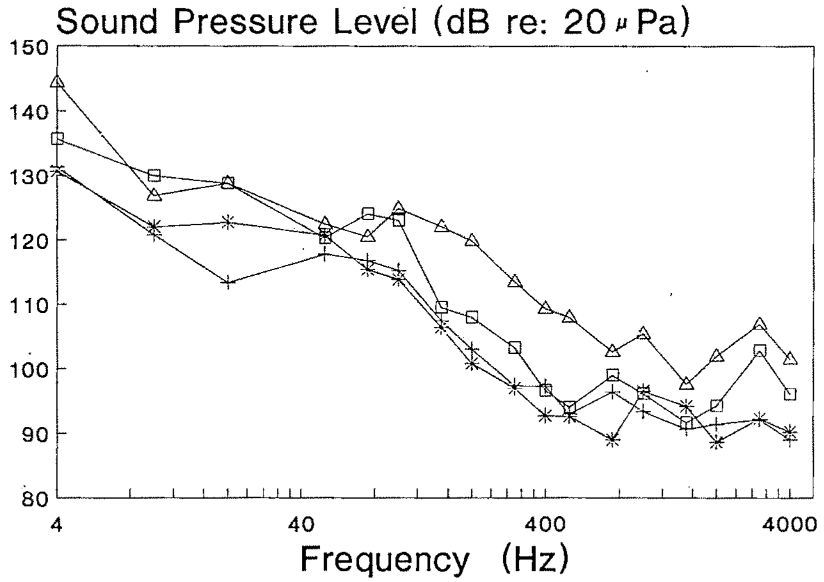

Fig. 3. Average intrauterine sound pressure levels recorded with hydrophone at different depths, generated by sinusoidal pure-tone vibration. △, 5 cm; □, 10 cm; +, 15 cm; *, 20 cm.

hydrophone depth and the sound pressure level produced ($p < 0.01$). The mean level dropped from 114 to 104 dB as the distance was increased from 5 to 20 cm. There was no observed interaction between frequency and distance ($p = 0.56$); that is, the observed drop with increasing distance was no greater at high frequencies than at low frequencies.

There was only an average 1.9 dB difference between the linear sound pressure level and that at the fundamental frequency, indicating that most of the transmitted vibrational energy was contained in the fundamental frequency. This finding was constant across frequencies. Sound pressure level measured at the first harmonic also decreased significantly as the frequency of vibration increased ($p < 0.0001$). The difference between the sound pressure level at the fundamental frequency and at the first harmonic became increasingly greater as the frequency of the input increased ($p < 0.0001$).

Comment

This study was designed to investigate the effects of vibration frequency and thickness of intervening maternal tissues on intrauterine sound levels produced by vibroacoustic devices applied to the abdomen. Knowledge about factors such as these will aid in the development of a vibroacoustic stimulator that produces intrauterine sound levels that are reproducible from one pregnancy to another and are within a desirable intensity range.

The characteristics of the ideal vibroacoustic stimulator have not yet been determined. Studies by Kisilevsky et al.[2] and Yao et al.[3] have shown that there is an incremental response of fetuses to stimuli of increasing intensity. It stands to reason that a fetal response test will be more interpretable if the intrauterine sound levels produced by a vibroacoustic device are similar in different women. If a given stimulator produces unusually high intrauterine sound levels in some women, their fetuses may respond with unphysiologic state changes of uncertain significance. If, on the other hand, a given stimulator produces sound pressure levels of insufficient intensity to elicit the desired state changes in the fetus, the test could inappropriately be interpreted as abnormal.

Previous clinical studies of vibroacoustic stimulation have not adequately addressed the question of intrauterine sound levels.[4, 5] The electronic artificial larynx is the stimulus source most commonly used in clinical practice, apparently because of its low cost, portability, and availability, not because of scientific evidence demonstrating that the type of stimulus it produces is ideal for its intended purpose. It may, in fact, produce too intense a stimulus, as demonstrated by Visser et al.[6] They showed that stimulation with the electronic artificial larynx produced unphysiologic disorganized states in the fetus, rather than bringing about a change from state 1F to 2F as is seen in a spontaneously reactive nonstress test. The current study confirms the previous work by Gerhardt et al.[1] showing that sound pressure levels produced by the electronic artificial larynx in the sheep uterus are much higher than those measured in air and quoted in the largest clinical studies.[7, 8] Since the electronic artificial larynx is a vibromechanical device designed to transmit sound pressure more efficiently through tissue and fluid than through air,[9] the determination that it produces approximately 82 dB sound pressure level measured at 1 m in air[7, 8] hardly seem relevant to estimating intrauterine levels.

Gerhardt et al.[1] showed in an earlier study that the intrauterine sound pressure level in the ewe varied from an average of 135 dB directly over the hydrophone to 101 dB when the electronic artificial larynx was moved 6 inches laterally on the skin surface. In this study we showed that with the electronic artificial larynx remaining fixed over the hydrophone, increasing the depth of the hydrophone from 5 to 20 cm decreased the mean sound pressure level from 119 to 102 dB. The difference in the maximum sound pressure level in the two studies is probably explained by the fact that in the earlier study the center point was determined by searching for the spot producing the highest sound levels, whereas in the current study it was determined from the geometry of the hydrophone stand (Fig. 1). Also, the depth of the hydrophone was <5 cm in the previous study. The clinical implication of these studies is that, in attempting to achieve a reproducible intrauterine stimulus, the thickness of the intervening tissues does not appear to be as important a factor as the location of the vibroacoustic device on the skin surface. This finding needs to be replicated in human studies.

Sound transmission loss through solid materials has been well characterized by structural engineers.[10] In general, energy in the form of sound or vibration is transmitted through solids more readily at lower than at higher frequencies. Sound transmission loss through animal tissues has been thoroughly studied for ultrasonic frequencies,[11] but there is very little information available on the transmission of vibration in the audible and subaudible frequency range. The complexity of the abdominal wall and organs in large animals, with varying resistive and resonant properties of the different tissues, makes precise predictions of intrauterine sound pressure levels from externally applied vibration impossible. However, as expected by the generally observed behavior of vibration in solids, intrauterine sound pressure levels generated with a vibration exciter was found to be greater at low frequencies. The clinical significance of this finding is that if a high-frequency stimulator is used for the vibroacoustic stimulation test, more voltage will have to be applied to achieve the same sound pressure levels as those achieved at low frequencies.

In normal pregnancy the uterus also is occupied by a fetus and placenta of varying dimensions that may reflect, absorb, or transmit vibrations. It should be noted that the experimental design we used, in which the fetus was removed, may result in different absolute intrauterine sound levels compared with those of a live fetus and an intact pregnancy. However, in a live animal the same progressive fall in sound levels with increasing depth and frequency would be expected.

We expected to find that with progressively increasing distances between the vibration exciter and hydrophone, sound pressure levels would fall off much more at high frequencies than at low frequencies. We postulated that a low-frequency vibroacoustic stimulator would therefore result in a more reproducible intrauterine stimulus, at varying distances, than a high-frequency device. Our results did not support this hypothesis, since the difference between the sound pressure levels at 5 and 20 cm were no greater at high frequencies than at low frequencies. Contrary to our expectations, our experiments did not support the idea that in the clinical application of a vibroacoustic stimulation test low-frequency stimuli would result in more reproducible intrauterine sound pressure levels. Our results may have been different if, rather than increasing the distance from the shaker to the hydrophone by moving the hydrophone within the abdominal cavity, we had used a model where the actual thickness of the abdominal wall was increased. Investigations in humans, with subjects of varying degrees of obesity, are needed to further evaluate possible interaction between vibration frequency, abdominal wall thickness, and intrauterine sound pressure levels.

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The interaction of electrosurgical bipolar forceps and generators on an animal model of fallopian tube sterilization

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Electrosurgical tubal sterilization with bipolar forceps has shown a variable and, in certain studies, a high failure rate. Some physicians have questioned if failure rates are due to an incompatibility between bipolar forceps and bipolar generators of different manufacturers. This manuscript presents data on tissue desiccations performed on a rat uterine horn model that uses two bipolar forceps of quite different designs and two popular bipolar generators. The data demonstrate that if an appropriate amount of energy, between 100 and 117 J, is applied to the tissue, consistent destruction occurs, regardless of the generator-forceps combination used. Histologic examination of the desiccated uterine horns displayed complete occlusion of all samples 8 weeks after the procedure. However, immediately and at 3 weeks after the procedure patent uterine horns were found. In the patent samples most samples displayed considerable electrosurgical damage. (AM J OBSTET GYNECOL 1991;165:443-9.)

Key words: Bipolar, electrosurgical, sterilization

During the last two decades, radio-frequency electrosurgical sterilization through the laparoscope has become a popular method for permanent female sterilization. Initially the procedure was performed by desiccation of several segments of the fallopian tube by monopolar electrosurgical forceps. When the procedure is performed in this fashion, the current flows from both tines of the forceps through the fallopian tube to a return electrode on the buttocks or thigh of the patient. Although this method is effective in producing permanent sterilization, persistent complications, including return electrode burns and alternate site burns in the abdominal cavity, e.g., bowel burns,[1-4] led Corson et al.[5] to suggest substituting bipolar forceps for monopolar forceps in electrosurgical laparoscopic sterilization.

In bipolar electrosurgery the radio-frequency current flows from one tine through the tissue to the second tine; this eliminates the return electrode with all the associated return electrode burns and virtually all alternate site burns. With the goal of improving patient safety, several researchers and companies introduced bipolar laparoscopic forceps,[6, 7] and the use of these various forceps has gained acceptance. Most groups have reported pregnancy rates similar to those after monopolar sterilizations[8]; however, several groups have reported an unexpectedly high incidence of pregnancy, from 4%[9] to 16%,[10] after bipolar sterilization.

Speculation as to the cause of these failures includes the low voltage of a bipolar output causing incomplete tissue destruction,[9] delayed fibrosis and tubal occlusion in bipolar procedures,[10] and generator-to-forceps mismatch.[11] This manuscript presents data showing that if sufficient energy is delivered into the tissue, successful tissue destruction can be accomplished, regardless of which generator or forceps combination is used.

Methods

The rat uterus was chosen to approximate the human fallopian tube. Rats possess a bifid uterus, each horn of which is the same approximate diameter as the central section of a human fallopian tube. Nonpregnant Sprague-Dawley rats weighing between 275 and 300 gm were used in the study. Each horn of the uterus could be coagulated once without causing significant postsurgical adhesions. Guidelines for the care and use of experimental animals was institution approved.

Uterine horns were examined histologically at four postsurgery intervals: immediately, 3 weeks, 8 weeks, and 12 weeks. Each time-interval group was treated identically. The groups consisted of 32 rats. Equal numbers of rats in each group were treated with a different combination of electrosurgical generator-forceps: Valleylab SSE2L generator and Storz forceps, Valleylab SSE2L generator and Kleppinger forceps, Wolf generator and Storz forceps, Wolf generator and Kleppinger forceps. Therefore eight rats in each time-interval group had bilateral uterine horn desiccations per-

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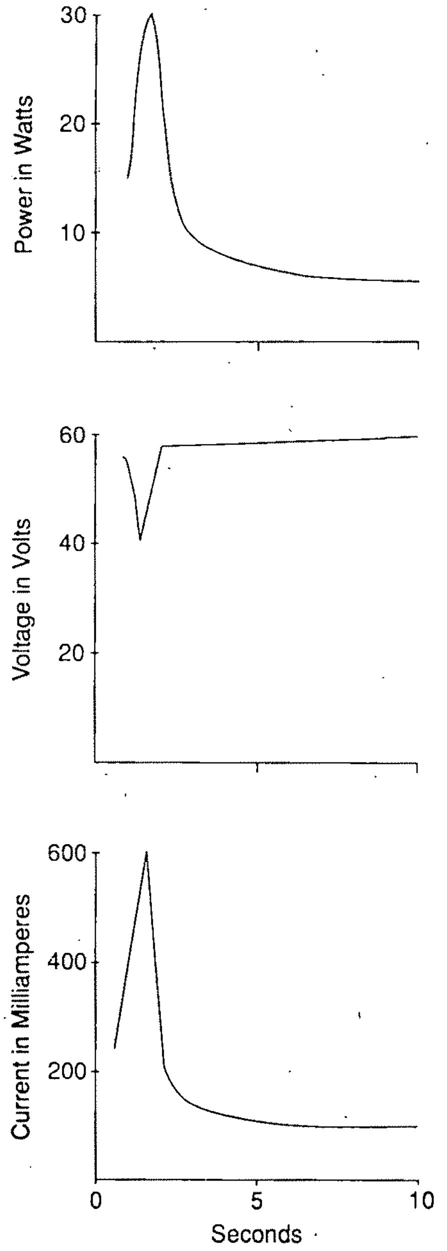

Fig. 1. Typical voltage, current, and power versus time tracings for bipolar uterine horn desiccation. Desiccation end point was visually determined by blanch, swell, and collapse.

formed with each of the four generator-forceps combinations. This resulted in 16 actual uterine burns to evaluate from each of the generator-forceps combinations.

The rats were anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). The uterus was viewed by a small midline incision. After each horn of the bifid uterus was desiccated once, the uterus was removed (immediate group) or the incision was closed with either staples or suture, and the animals were allowed to recover.

The tubal desiccations were performed with a single application of the forceps to each uterine horn. Power was applied continuously until the standard visual end point was reached. As the uterine horn desiccated, it would swell, blanch, and collapse with a dry appearance; at this point the power application was terminated. The visual end point was noted in all animals.

The technique of the application of the Kleppinger forceps to the uterine horn deserves comment. As designed, the flat duckbill tongs of the instrument grasp the mesosalpinx while the curved portion surrounds the tube. The intent is to destroy the tube and the associated blood supply simultaneously. Some physicians also recommend repositioning the forceps' duckbill over the previous tube desiccation site to deliver a second coagulation.[10, 12] Placing the curved portion around the uterine horn could not be performed on the rat as the flat duckbill tongs would short to each other without desiccating the tube, because the small amount of thin mesosalpinx-like tissue on the rat uterine horn. Therefore the flat duckbill tongs were placed directly on the horn for a single coagulation.

The Wolf generator output is a pure sine wave alternating current; therefore the Valleylab SSE2L was tested in the bipolar "cut" mode, which also has a pure sine wave output. The Wolf generator was used on a setting of 6 and the Valleylab generator at a setting of 4; these settings correspond to approximately 50 W power into a 100 Ω load. The electrosurgical generator voltage, the radio-frequency current supplied to the tissue, and the corresponding power delivered to the tissue were measured by a demodulator circuit, which was previously described.[13] The three values were measured over time and recorded on an oscillographic recorder. A typical set of voltage, current, and power traces is shown in Fig. 1. The power was not constant but varied with time; therefore the total energy delivered into the tissue was calculated by multiplying the measured power by the time, i.e., Energy = Power × Time (where the units are energy in joules, power in watts, and time in seconds).

The rats were killed, and the entire uterus was removed and fixed in 10% formalin for at least 24 hours. For the immediate group and the 3-week group, the desiccated segment and a normal segment of each uterine horn were embedded in paraffin. For the immediate group, cross sections of the uterine horn were examined; for the 3-week group, cross sections were cut; and for the 8-week group, longitudinal sections were cut. All histologic samples were stained with hematoxylin-eosin stain. The histologic sections were examined without knowledge of the burn technique by a surgical pathologist (J.A.B.).

The immediate group was examined for evidence of tissue damage in the uterine horn epithelium, muscle layer, and surrounding soft tissue. Vascular damage and fat necrosis also were noted. Four measurements were performed on each horn sample: the normal horn diameter, the normal lumen diameter, the desiccated horn diameter, and the desiccated lumen diameter. From these measurements the percentage decrease in the diameter of the horn and the lumen was calculated.

The 3- and 8-week groups were examined for the patency of the horn lumen, scarring of the muscle layer, and fat necrosis. The vessels were examined for thrombosis, occlusion, recanalization, reendothelialization, fibrinoid necrosis, and coagulation necrosis.

The remaining 12-week group was simply examined by gross dissection for loss of segment in the desiccated section of the uterine horn.

Results

Although the generators were set at the same approximate power settings, the oscillographic recordings of the power delivered to the tissue varied dramatically. Neither generator supplied a constant power to the tissue over time (Fig. 2). For a given generator-forceps combination the power delivered varied from uterine horn to uterine horn, some horns required low power for a long period of time whereas others required high power for a short period. The power variations were substantial: The Wolf generator maximum powers varied from 11.2 to 28.8 W, while the Valleylab generator maximum powers varied from 18.2 to 29.9 W. The variations both in maximum power and in power levels over application time necessitate the calculation of the total energy applied over the treatment period.

The total energy delivered to the tissue and the total desiccation time among the four groups evaluated did not statistically differ. Therefore the energies and desiccation times for the four time-interval groups were evaluated together for each generator and forceps combination. Thus there are 32 animals or 64 uterine horn burns in each of the four combinations, yielding a total of 256 horn desiccations. Comparisons of the average energy used for uterine horn desiccation were made for each of the four generator and forceps combinations (Table I): (1) Wolf-Storz versus Wolf-Kleppinger, (2) Valleylab-Storz versus Valleylab-Kleppinger, (3) Wolf-Storz versus Valleylab-Storz, and (4) Wolf-Kleppinger versus Valleylab-Kleppinger. The average time rquired to complete the desiccation to a visual end point also was compared for each of the four generator and forceps combinations. Of the eight energy and time comparisons, only three were statistically significant: (1) the Storz-Wolf versus the Kleppinger-Wolf energy comparison, (2) the Kleppinger-Wolf versus the Kleppinger-Valleylab energy comparison, and (3) the Kleppinger-Wolf versus the Kleppinger-Valleylab desiccation time comparison. Although the data display highly significant p values, the actual averages are relatively similar with variations of approximately 10% to 15% in total energy and only 1 second or 10% in application time.

In the immediate group, 16 uterine horns (eight animals) were examined for each of the four generator-forceps combinations. The data are summarized in Table II. The diameter of horn and lumen for normal and treated groups was measured, and the ratio of diameters for treated versus normal was calculated. In comparing the horn and lumen ratios for the Storz

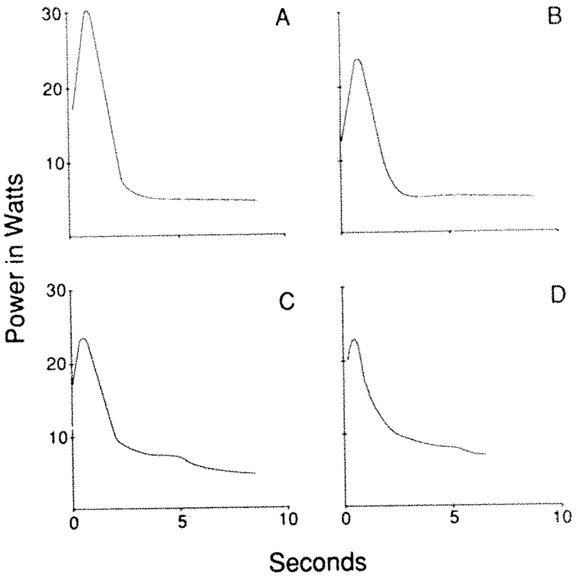

Fig. 2. Typical tracings of power versus time for bipolar uterine horn desiccations. **A,** Wolf generator and Kleppinger forceps; **B,** Wolf generator and Storz forceps; **C,** Valleylab generator and Kleppinger forceps; **D,** Valleylab generator and Storz forceps.

Table I. Energy and time for bipolar uterine horn desiccations

	Forceps		
Generator	*Storz*	*Kleppinger*	*p Value*
Wolf			
Energy (J)	117.1 ± 4.5	100.6 ± 2.6	0.001*
Time (sec)	10.0 ± 0.3	9.4 ± 0.2	0.062
Valleylab			
Energy (J)	114.7 ± 10.4	110.8 ± 2.7	0.20
Time (sec)	10.4 ± 0.2	10.2 ± 0.2	0.30
p Value			
Energy	0.34	0.004*	
Time	0.12	0.004*	

n = 64 uterine horns for each energy and time group.
*Statistically significant.

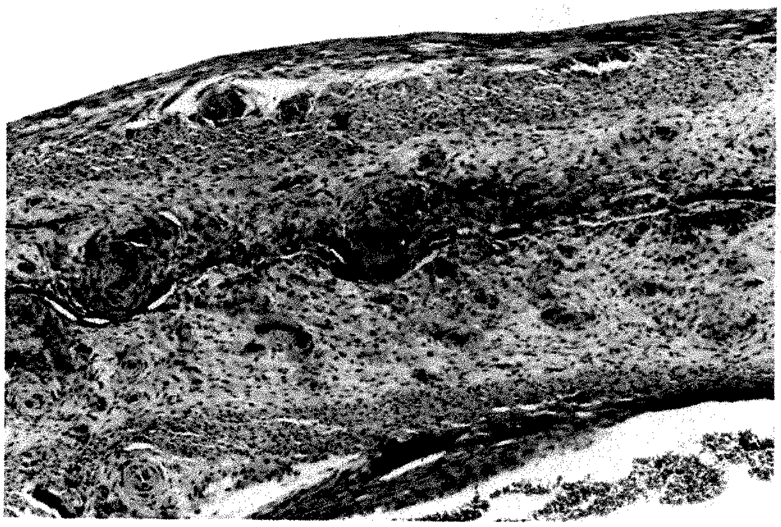

Fig. 3. Uterine horn showing extensive electrosurgical damage, particularly to epithelial lining, with total occlusion of lumen. Horn desiccation is from immediate group, performed with Valleylab generator and Storz forceps. (Hematoxylin-eosin. Original magnification × 16.)

Table II. Ratio of desiccated verus normal horn and lumen diameters in immediate group

Generator	*Ratio of diameters (desiccated/normal)*	*Forceps*		*p Value*
		Storz	*Kleppinger*	
Wolf				
Horn		0.72 ± 0.03	0.53 ± 0.04	0.001*
Lumen		0.68 ± 0.12	0.22 ± 0.06	0.002*
Valleylab				
Horn		0.72 ± 0.04	0.40 ± 0.05	<0.0005*
Lumen		0.54 ± 0.22	0.16 ± 0.08	0.056
p Value				
Horn		>0.40	0.022*	
Lumen		0.28	0.25	

n = 16 uterine horns for each horn and lumen ratio group.
*Statistically significant.

versus the Kleppinger forceps with the use of a given generator, three of the four comparisons were statistically significant at levels of $p < 0.002$, and the remaining combination tested at $p = 0.056$. The nonsignificant value, the lumen diameter comparison of the Storz versus the Kleppinger forceps with the use of the Valleylab generator, was strongly influenced by large variations in the treated lumen diameter; two horns showed increases in the lumen diameter after treatment.

Only one of the four comparisons between generators for a given forceps tested significant. This was the comparison of horn diameter with the use of the Kleppinger forceps for the Wolf versus the Valleylab generator, $p = 0.22$ (Table II). In the short term the Kleppinger forceps, used as described in the Methods section, displayed reduced tube and lumen diameter after treatment over the Storz forceps when used with either generator.

Of the 64 uterine horns in the immediate group, only 26 displayed complete occlusion (Fig. 3). However, all horns displayed full-thickness damage, including both epithelial and muscle damage as a result of the electrosurgical desiccation. The great majority displayed soft tissue damage, coagulated blood vessels, and necrosis in the surrounding fat. A typical patent horn with electrosurgical damage is displayed in Fig. 4. Both generators, as previously described, caused more acute damage with the Kleppinger forceps than the corresponding generator with the Storz forceps.

In the 3-week group, two of the 64 uterine horns that were examined histologically were patent. The pa-

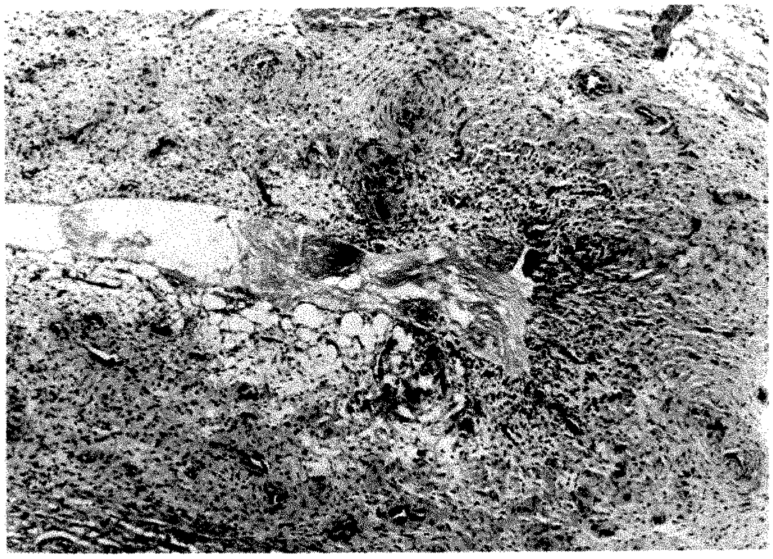

Fig. 4. Uterine horn showing extensive electrosurgical damage, especially in epithelial lining, with some residual lumen. This horn desiccation is from immediate group performed with Valleylab generator and Kleppinger forceps. (Hematoxylin-eosin. Original magnification ×16.)

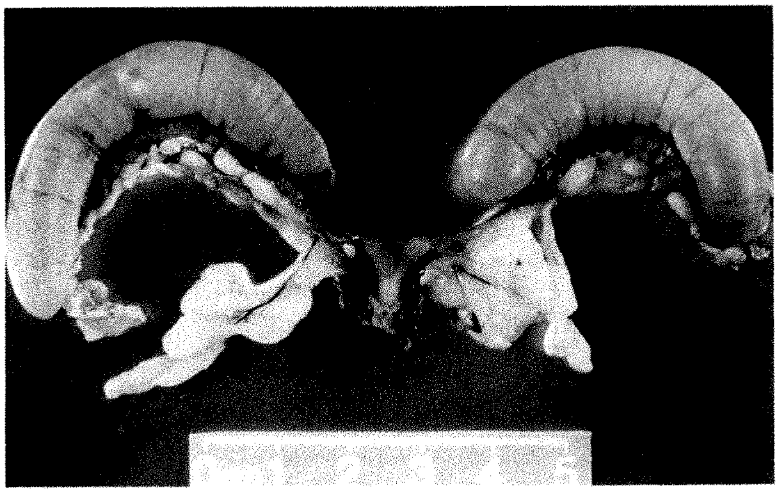

Fig. 5. Entire bifid uterus from animal in 12-week group. Both horns display missing segment at area of electrosurgical desiccation. Remainder of horn is dilated.

tent horns had desiccations performed with (1) the Kleppinger forceps and the Valleylab generator and (2) the Storz forceps and the Wolf generator. The tissue section from the Kleppinger-Valleylab combination did show muscle scarring, fat necrosis, and vessel fibrinoid necrosis. The section from the Storz-Wolf combination displayed little destruction, either grossly or histologically. In both of these cases the horn on the opposite side was identified as not patent.

In the 8-week group, all 64 gross samples displayed a loss of a segment of uterine horn, which is assumed to have undergone resorption. No histologic sample displayed patency. Procedure energies and times were not statistically different from those of the other three treatment groups.

The remaining 12-week group was examined grossly (Fig. 5). In all cases a segment of the uterine horn was absent and was assumed to have undergone necrosis and resorption. The average energies and desiccation times were virtually identical to those of the immediate, 3-week, and 8-week groups. In all cases except one, both ends of the horn were stenotic and closed. The exception was a desiccation performed with the Kleppinger-Wolf combination. In this case the treated area was absent, but one end of the surviving horn was patent with a relatively normal-appearing lumen. The adjacent end was closed.

Comment

While use of bipolar electrosurgical tubal sterilization has decreased associated burn complications, many groups have reported an increased incidence of sterilization failure. One explanation of these failures has been the mismatch of forceps and generator. Should a given vendor combination be truly matched, the substitution of another generator or forceps would present important medical and legal complications and less predictable surgical outcome.

The data presented in this manuscript suggest little clinical importance to generator-forceps combination. While statistical differences were shown in the total energy required and the length of time required for complete visual desiccation, the differences were only 10% to 15%. The largest average time variation was only approximately 1 second.

Although both generators were set for approximately 50 W output power, the maximum power output during the procedures was <30 W for both generators. This is a result of the generator output power being specified by the manufacturer into a constant value pure resistance. The tissue, however, is not a pure resistance but has a component of capacitance as a result of cell membranes. (Capacitors store energy.) The value of tissue resistance is also constantly increasing during the procedure as the tissue desiccates. The data demonstrate that 11.2 to 29.9 W was adequate to provide the needed tissue destruction; however, lower powers required longer application times.

The data demonstrated that most horns were patent immediately after a procedure, regardless of which generator-forceps combination was used, although the damage in all samples was extensive. At 3 weeks two of 64 horns were patent. One of these patent samples displayed extensive damage, whereas the other sample displayed little damage. At 8 weeks all lumina were closed. In the 12-week group one horn had a patent distal end, a relatively normal-appearing lumen, and a closed proximal end. With the assumptions that the extensively damaged horn from the 3-week group would ultimately fibrose and close and that the patent horn in the 12-week group was nonfunctional because the proximal end was closed, the only failure would be the horn from the 3-week group that displayed little damage. This would represent only one failure in the series of 256 uterine horn desiccations, for a failure rate of 0.4%. If all three of these horns were considered failures, the rate would be 1.2%. Thus a failure rate, although small, can be expected, even with accurately measured energy delivery. This finding underscores the need for alternative contraceptive methods for at least 8 weeks after a procedure.

The various forceps did display differences in their ability to reduce both the horn and lumen diameter immediately after a procedure. The Kleppinger forceps significantly reduced both horn and lumen diameter when used with either generator. This is likely a result of the compressing action of the wide duckbill that was applied directly to the uterine horn, as previously described in Methods.

The data presented suggest no necessity to match a particular generator to a particular forceps and that successful tissue destruction can be performed with any of the generator-forceps combinations. The radio-frequency currents produced by bipolar forceps are confined to flow over a smaller tissue volume than are those of monopolar forceps. Therefore adequate tissue injury would require a longer current application time. One can only speculate that failure to apply radio-frequency current for a sufficient amount of time could lead to incomplete destruction in the reported failures.

Our data demonstrated that the visual end point was reproducible in both measured time and energy. However, in laparoscopic procedures the clinical end point of "blanch, swell, and collapse" may be more difficult to evaluate than in our open surgical model. To achieve tissue destruction, radio-frequency current must be passed for an adequate time period. Without a good visual end point, defining an adequate time period becomes difficult. At present, there is no clinical or electrical option for measuring energy delivered to the tissue. The Wolf generator provides an ammeter to monitor the radio-frequency current. However, Wolf recommends that the meter be used to test electrical continuity, i.e., to test that the circuit is complete. Some physicians use the circuit to monitor the end point of the tube desiccation; however, this technique is not without potential failure. It is possible to monitor current flow through the meter without flow through the tissue; this could occur if the forceps tines short or touch through a hole in the mesosalpinx. It is also possible for the meter to show reduced current flow without complete tube desiccation by retaining small amounts of dried tissue on the forceps from a previous application. A true measure of complete tube desiccation would require monitoring changes in either the tissue resistance or the energy delivered to the tissue; nothing commercially available will perform this function.

Many variables also affect the time required to achieve adequate tissue destruction. Lower power settings will require longer generator activation time than will higher power settings. Coagulation and blend waveforms produce less power at a given setting than does a cut waveform and thus require longer activations. At a given power setting thicker tissue also will require longer time periods. Differences in blood flow and tissue composition also will affect the rate of tissue desiccation. With these potential variables and, at present, a visual end point as the measure of adequacy, it is important to apply radio-frequency current to several contiguous sites to achieve adequate tissue destruction.

The application of the Kleppinger forceps also deserves comment. The duckbill portion was designed to desiccate the mesosalpinx containing the blood supply to the fallopian tube. The curved forceps portion is designed to surround the tube and apply simultaneous current. During this typical application it is possible that the flat duckbill portion becomes electrically shorted and the majority of the current passes between the flat tines. Subsequently little current passes through the fallopian tube, thus resulting in inadequate tissue destruction. Therefore we agree with the recommendation that an additional desiccation of the fallopian tube with the flat duckbill section be applied after the initial simultaneous current application to both tube and mesosalpinx. This procedure has previously been advocated by Kleppinger.[14]

Our data show that hardware available for electrosurgical bipolar tubal sterilization is adequate to apply sufficient power for an adequate amount of time (e.g., total energy) to destroy tissue of similar composition and size of the human fallopian tube.

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Fetal hypertension induced by norepinephrine infusion and umbilical artery flow velocity waveforms in fetal sheep

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This study was designed to examine the effects of fetal hypertension on the umbilical artery pulsatility index. Fetal arterial blood pressure and umbilical venous pressure were measured in eight sheep, 3 to 5 days after surgery. Umbilical blood flow was measured with an electromagnetic flowmeter around the common umbilical vein. Umbilical artery flow velocity waveforms were obtained either by an indwelling 5 MHz pulsed Doppler device ($n = 4$) or transcutaneously by a 4 MHz continuous-wave Doppler device ($n = 4$). Fetal blood pressure was raised by intravenous infusion of norepinephrine 10 μg/min during 5 minutes. Norepinephrine infusion resulted in elevated arterial and umbilical venous pressures, accompanied by a bradycardia during the first 3 minutes. Umbilical blood flow, calculated placental vascular resistance, and umbilical artery pulsatility index did not change. After atropine administration, the norepinephrine-induced elevated arterial and umbilical venous pressures were accompanied by tachycardia, increased umbilical blood flow, and no change in placental vascular resistance and umbilical artery pulsatility index. It is concluded that fetal arterial hypertension provoked by norepinephrine infusion has no effect on placental vascular resistance, umbilical blood flow, and umbilical artery pulsatility index. (AM J OBSTET GYNECOL 1991;165:450-5.)

Key words: Placental vascular resistance, norepinephrine, infusion of; pulsatility index, umbilical artery; hypertension, fetus

Since the introduction of Doppler ultrasonography in the study of the fetal circulation by Fitzgerald and Drumm[1] more than a decade ago, an overwhelming number of articles about umbilical artery flow velocity waveforms have been published. Initial reports showed promising results concerning the capability of umbilical artery velocimetry to detect intrauterine growth restriction and to predict fetuses with poor perinatal outcome.[2-5] Later studies questioned the validity of umbilical waveform analysis with regard to the detection of intrauterine growth restriction and compromised fetuses.[6-9] In a case of an abnormal umbilical artery waveform, it is suggested that the fetus may be seriously ill. On the other hand, a seriously ill fetus may have a normal umbilical artery waveform.[7] Abnormal waveforms can be seen already in growth-retarded fetuses several weeks before the occurrence of fetal heart rate decelerations as a sign of fetal hypoxia.[2] In contrast, we have shown in fetal sheep that moderate and severe fetal hypoxia can exist without any effect on umbilical artery waveform indices.[10-13] Therefore analysis of the umbilical artery waveform does not seem to give adequate information as to what extent fetal reserve capacity and compensatory mechanisms are mobilized. This view is supported by a recent article of Block et al.,[14] in which study the observed distribution of regional blood flow in fetal sheep was not different during imposed acute hypoxemia in fetuses with normal placentas compared with fetuses in which intrauterine growth restriction was created by umbilical embolization. Hypoxic stress results in a release of catecholamines, mainly norepinephrine, which has a predominant α-adrenergic receptor–stimulating effect. Through α-receptor stimulation, fetal cardiac output redistribution occurs, which is essential for an adequate supply of oxygen to high-priority organs such as brain and heart during hypoxemia.[15, 16] Norepinephrine infusion causes an increase of systolic, diastolic, and mean arterial pressure in the fetal circulation,[17, 18] with secondary effects on fetal heart rate or umbilical blood flow.[19] The influence of fetal hypertension on the umbilical artery velocity waveform is not known. The aim of this study was to investigate the effect of norepinephrine-induced increased fetal arterial blood pressure on the umbilical artery flow velocity waveform. Fetal blood pressure was raised by intravenous infusion of norepinephrine. The umbilical artery flow velocity waveform was described by the pulsatility index, according to Gosling et al.[20]

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Material and methods

Surgery. The experiments were carried out in eight ewes of the Dutch Texel breed, 3 to 5 days after surgery, between 113 and 124 days' gestation (term, 147 days). Surgical instrumentation was carried out under aseptic conditions and with the animal under general anesthesia, induced with pentobarbital sodium and maintained with 1% halothane in a 2:1 mixture of nitrous oxide and oxygen, and involved insertion of a catheter in the distal aorta through a femoral artery, a catheter in a femoral vein for drug infusion, a catheter in a major umbilical vein through a cotyledonary vein, placement of an electromagnetic flowmeter around the intraabdominal common umbilical vein, and placement of an inflatable balloon occluder around the umbilical cord at the site of the abdominal insertion for in vivo zero blood flow calibration. A 5 MHz pulsed Doppler device was placed around one of the two umbilical arteries. Anesthetic technique and surgical instrumentation has been extensively described previously.[10, 21] The surgical and experimental procedures were approved by the local animal medical ethics committee.

Measurements. Blood flow in the common umbilical vein was measured with a Skalar Transflow 601 flowmeter system (Skalar, Delft, The Netherlands). Fetal arterial blood pressure and umbilical venous pressure were determined with the zero point at the level of the ewe's spine. Fetal heart rate was calculated from the pulse of the arterial blood pressure wave. All signals were led to amplifiers (Hewlett Packard 8800 series, Hewlett Packard, Andover, Mass.), displayed on a monitor and an eight-channel strip chart recorder, stored on magnetic tape, digitized, and analyzed with a computer. Umbilical artery waveforms were obtained by the 5 MHz pulsed Doppler probe around one umbilical artery in four animals. Because of malfunctioning of the 5 MHz indwelling Doppler probe, umbilical artery velocity waveforms were obtained by a 4 MHz continuous-wave Doppler device (Delalande Electronique, Courbevoie, France) in the other four animals. The ultrasonographic beam was insonated from the abdominal skin of the standing ewe after visualization of the umbilical cord by ultrasonography (Scanner 400, Pie Medical, Maastricht, The Netherlands). The origin of the received signals was verified by comparison with fetal heart rate, derived from the arterial pressure waveform, and by presence of oppositely directed venous blood flow velocities within the Doppler sample volume. The Doppler frequency shift signals were stored in quadrature on analog tape, synchronously with the other signals for off-line analysis. After transformation of the Doppler shift signals by a spectrum analyzer (Doptek Ltd., Chichester, England), pulsatility index values were obtained by drawing the maximum envelope curves of the frequency-shift spectra with a hand-guided light pen. In the group of fetuses in which the continuous wave Doppler device was used, it was not always possible to obtain representative umbilical artery velocity waveforms at every point of time. Nevertheless, velocity waveforms were obtained in all animals at 4 and 5 minutes of infusion.

Experiments. The animals were allowed to recover for 3 to 5 days after surgery. After baseline conditions were established during 1 hour, norepinephrine was infused by an infusion pump (No. IP3H; Vicker Ltd., Basingstoke, England) at a rate of 10 μg/min during 5 minutes in eight fetal sheep with the autonomic nervous system intact (group 1). To eliminate the baroreceptor effect of the norepinephrine-induced hypertension on the fetal heart rate, cholinergic blockade was established by atropine at a dose of 1 mg/kg estimated fetal weight (group 2). Each animal was used in two protocols, one without atropine and one after atropine administration on different days after randomization. Ten minutes before and 10 minutes after the experiments an arterial blood sample was drawn for analysis of fetal P_{O_2}, P_{CO_2}, and pH.

Calculations and data analysis. The mean values for the hemodynamic variables were assessed over 10-second periods, at 1 minute before infusion (control period), at every minute during the infusion, and at 5 minutes after the infusion. Mean umbilical artery pulsatility index was calculated over five consecutive heart cycles that occurred during these 10-second intervals. Placental vascular resistance was defined as the difference between fetal arterial pressure in the distal aorta and venous umbilical pressure divided by umbilical venous blood flow. Statistical analysis was performed for each variable on the pooled values from each animal by comparing the control values before infusion with the values during and after the infusion by means of a paired Student *t* test with Bonferroni correction for repeated analyses.[22] In those cases in which umbilical artery velocity waveforms, at the various points of time, could not be obtained in all eight animals, comparisons were made between the reduced number of values during the infusion period and their respective control values. A p value of <0.05, after Bonferroni correction, was taken to represent statistical significance. The values of the fetal arterial acid-base balance were compared before and after norepinephrine infusion with a paired Student *t* test. Mean values ± SE are reported.

Results

Intact autonomic nervous system (group 1). The absolute values of the fetal cardiovascular variables during the control period for the animals with intact autonomic nervous system (group 1) and after atropine administration (group 2) are shown in Table I. Fig. 1 shows the effects of norepinephrine infusion on the

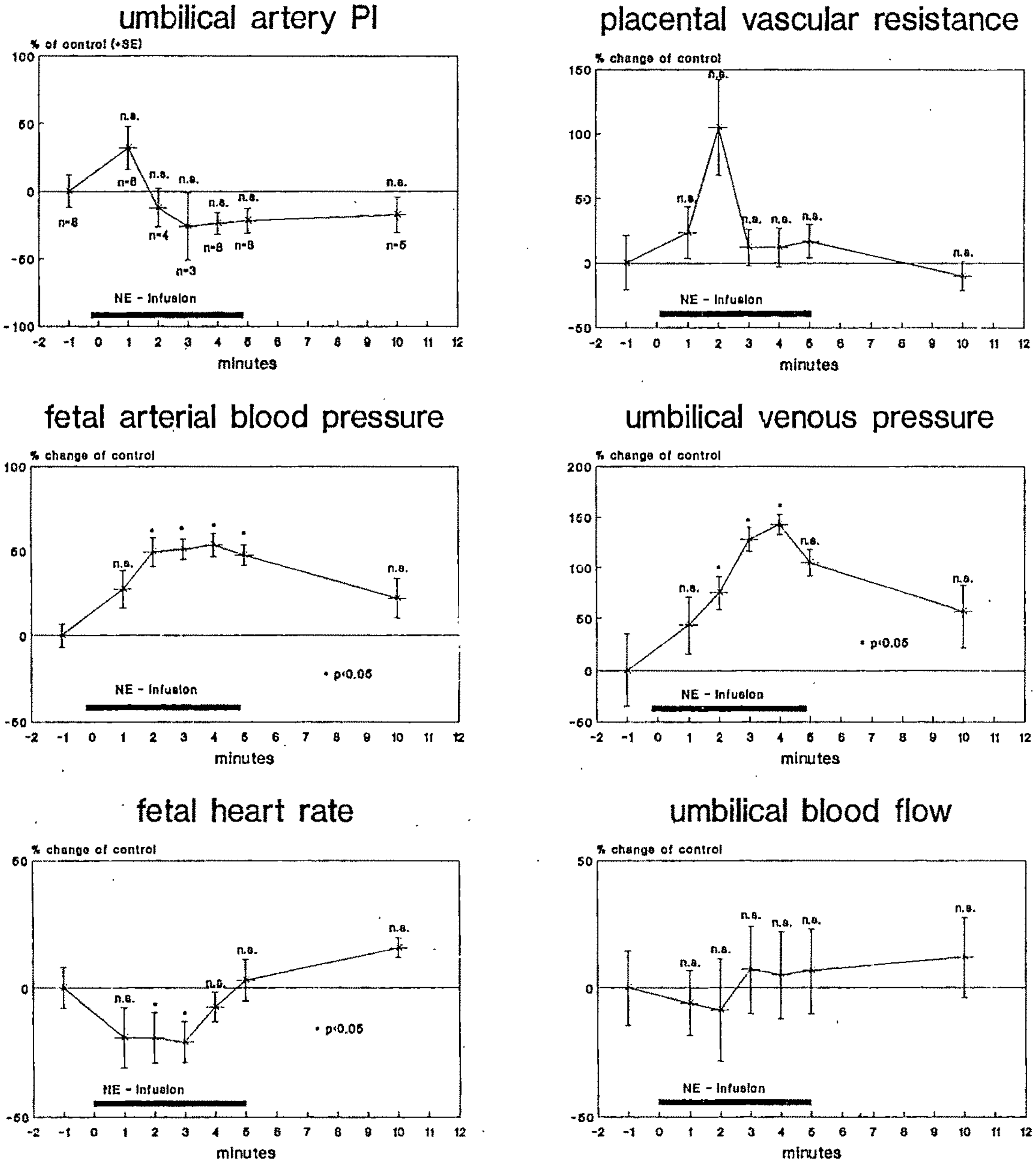

Fig. 1. Cardiovascular variables and umbilical artery pulsatility index before, during, and after 5 minutes of norepinephrine infusion in eight fetuses. Values are expressed as percent change of control (group 1).

fetal cardiovascular variables, expressed as percent change from control value.

Norepinephrine infusion caused an increase in mean arterial blood pressure. The increase in arterial pressure was accompanied by a bradycardia that lasted during the first 3 minutes of infusion. Fetal bradycardia had disappeared at 4 and 5 minutes during infusion and 5 minutes after infusion. Umbilical venous pressure significantly increased at 2, 3, and 4 minutes during infusion. Placental perfusion pressure was not significantly elevated during norepinephrine infusion. Umbilical blood flow also did not change. Placental vascular resistance did not change significantly during and after infusion, compared with baseline values. A sufficient number of good-quality umbilical artery blood flow velocity waveforms were obtained for statistical analysis at 1, 4, and 5 minutes of infusion.

After an initial nonsignificant rise in umbilical artery pulsatility index, a tendency toward a decrease was observed during the norepinephrine infusion. The changes, however, were not significant with Bonferroni correction at 1, 4, and 5 minutes of infusion.

After parasympathetic blockade with atropine (group 2). Fig. 2 depicts the results of norepinephrine infusion on the fetal cardiovascular variables after cholinergic blockade by atropine, expressed as percent change from control (Table I). Norepinephrine infusion caused a more pronounced increase in mean arterial pressure, as in the experiments without cholinergic blockade. Fetal heart rate, however, significantly increased during norepinephrine infusion. Umbilical venous pressure increased significantly and returned to baseline values 5 minutes after infusion. Placental perfusion pressure also significantly increased during and after infusion. A significant rise in umbilical blood flow occurred during infusion and was still present at 5 minutes after infusion. Placental vascular resistance did not change during or after infusion. The umbilical artery pulsatility index tended to decrease during the infusion, but the change was not significant. The number of observations of umbilical artery pulsatility index at 1, 2, and 3 minutes of infusion and 5 minutes after infusion were from six, seven, seven, and six fetuses, respectively.

No significant changes in fetal acid-base balance were seen after norepinephrine infusion. Fetal arterial pH was 7.35 ± 0.01 before and 7.31 ± 0.01 after infusion, whereas P_{CO_2} was 5.72 ± 0.30 and 5.73 ± 0.29 kPa, respectively, and P_{O_2} was 1.89 ± 0.25 kPa before and 2.27 ± 0.30 kPa after norepinephrine infusion.

Comment

The purpose of this study was to investigate the effect of fetal hypertension on the umbilical artery flow velocity waveform expressed by the pulsatility index.

Norepinephrine infusion caused arterial hypertension in the fetal circulation, which is in accordance with results of previous studies.[16-19, 23, 24]

The increase in mean arterial pressure had a considerable effect on fetal heart rate in fetuses with the autonomic nervous system intact. Because of baroreflex activity fetal heart rate decreased during the first 3 minutes of infusion but recovered at 4 minutes of infusion. To avoid reflex bradycardia during fetal hypertension, another series of norepinephrine infusions was performed after cholinergic blockade with atropine (group 2), because it is known that bradycardia increases the umbilical artery pulsatility index.[10] A second reason for parasympathetic blockade is the fact that calculation of placental vascular resistance is questionable during bradycardia, because a concomitant decrease in umbilical blood flow during bradycardia cannot be attributed to an increase in vascular resistance.[19, 23] The increase in fetal heart rate in group 2 during norepinephrine infusion is explained by the prevailing β-adrenergic system, because parasympathetic vagal activity is blocked.

Table I. Hemodynamic control variables before norepinephrine infusion in group 1 without atropine and group 2 after atropine administration

Variable	*Control (group 1) (mean ± SE)*	*Control after atropine (group 2) (mean ± SE)*
Umbilical artery pulsatility index	0.99 ± 0.12	0.82 ± 0.14
Fetal heart rate (beats/min)	192 ± 15	222 ± 16
Fetal blood pressure (mm Hg)	43.3 ± 2.9	40.3 ± 4.6
Umbilical venous pressure (mm Hg)	12.4 ± 4.3	15.3 ± 5.6
Fetal blood pressure − Umbilical venous pressure (mm Hg)	30.9 ± 3.6	25.0 ± 2.3
Umbilical blood flow (ml/min)	408 ± 59	335 ± 40
Placental vascular resistance (mm Hg · min · ml^{-1})	0.089 ± 0.019	0.076 ± 0.010

During norepinephrine infusion a tendency of the umbilical artery pulsatility index to decrease was noted in group 1 and in group 2. These changes, however, were not significant after the Bonferroni correction for repeated analyses. In group 1 placental vascular resistance did not change, mainly because of the relatively small increase in perfusion pressure with rather stable umbilical blood flows. In group 2 placental perfusion pressure and umbilical blood flow both increased. Therefore no change in calculated placental vascular resistance occurred. In contrast to the results in group 1, the umbilical arterial-venous pressure difference was more pronounced after cholinergic blockade, and it paralleled the increase in umbilical blood flow and fetal heart rate. The increase in umbilical blood flow in group 2 was very likely related to the increase in fetal heart rate.[23]

Furthermore, norepinephrine infusion caused a significant rise in umbilical venous pressure in both groups. The increase in umbilical venous pressure was more gradual than the increase in arterial pressure, which explains the increase in perfusion pressure during the first 2 minutes of infusion in group 1. The later onset of the umbilical venous pressure increase also was reflected in the higher mean values of calculated pla-

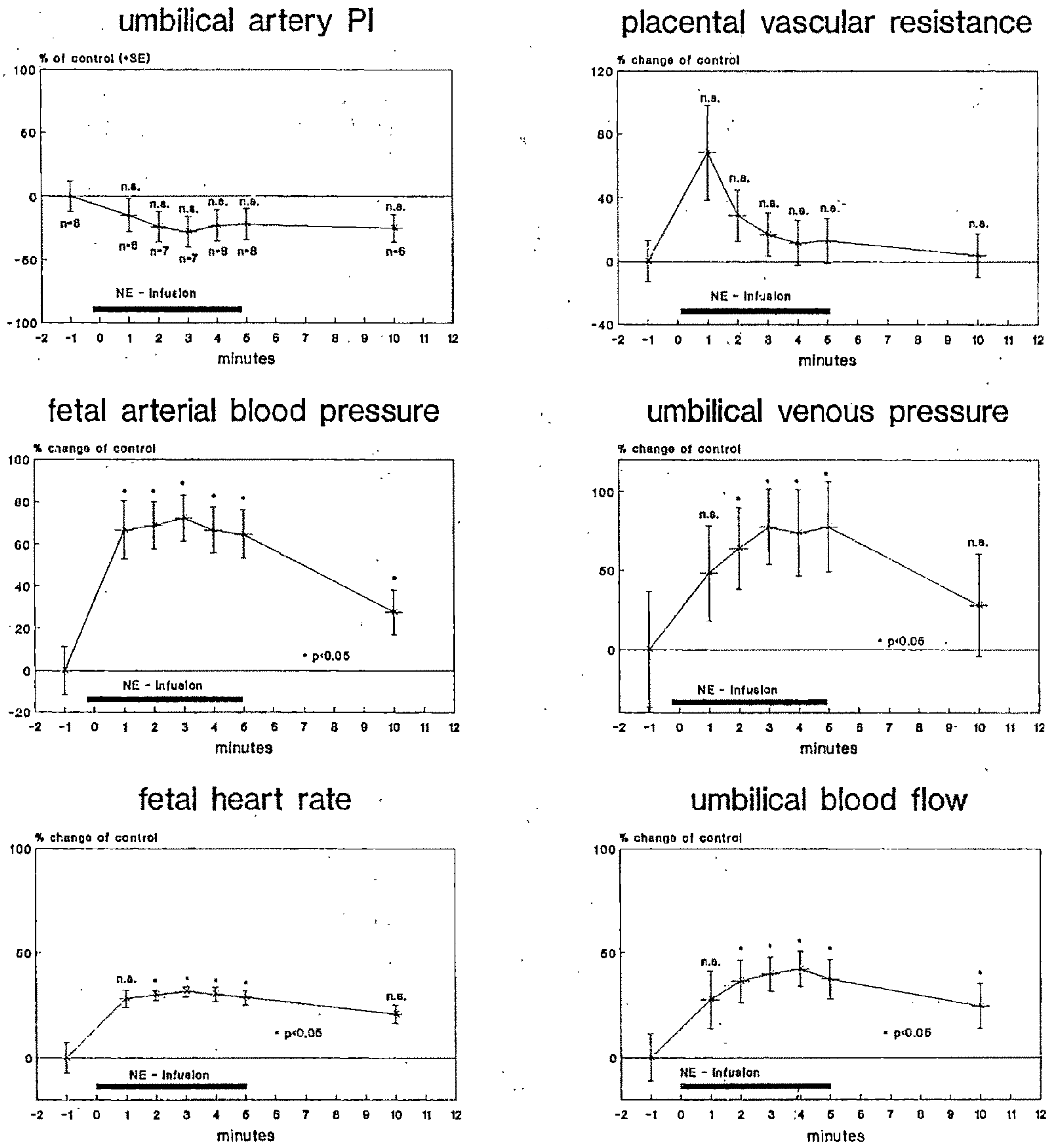

Fig. 2. Cardiovascular variables and umbilical artery pulsatility index after cholinergic blockade by atropine before, during, and after 5 minutes of norepinephrine infusion in eight fetuses. Values are expressed as percent change of control (group 2).

cental vascular resistance during the first 2 minutes of infusion.

Norepinephrine constricts the systemic vascular bed while the umbilical arteries are unaffected. It follows that in cases of catecholamine release, as during hypoxic stress, the fetus is capable of regulation of placental pefusion pressure, not only by increasing arterial blood pressure but also by increasing umbilical venous pressure, to maintain umbilical blood flow and avoid compromise of gas exchange. This is illustrated by the fact that pH, Pco_2 and Po_2 were not affected, which is in accordance with previous reports.[16, 23] We infer that umbilical venous vasomotor tone may play an important role in regulating placental pefusion pressure and umbilical blood flow.

Inferior vena cava pressure is not representative for umbilical venous pressure, since it is only moderately affected by norepinephrine infusion.[24, 25] Placental vascular resistance calculations using vena cava pressure may therefore increase the numerator and hence increase calculated placental vascular resistance.[17, 18] Adamson et al.[24] reported no significant changes in resistance of the umbilical artery and intrinsic cotyledon vasculature during norepinephrine infusion and no change in umbilical blood flow in an acute experiment. They observed, however, a rise in umbilical venous resistance, from which they concluded that the umbilical vein was constricted during norepinephrine infusion. Recently Paulick et al.[26] observed a pressure drop in the umbilical vein, from the placenta to the portal sinus, which increased during hypoxemia, indicating increased resistance. This would distend and recruit vessels in the placenta and hence improve gas exchange.

It is concluded that:

1. Fetal hypertension caused by norepinephrine infusion has no effect on umbilical artery pulsatility index.
2. Norepinephrine infusion does not alter placental vascular resistance.
3. Norepinephrine infusion has no effect on umbilical blood flow.
4. Norepinephrine infusion increases umbilical venous pressure.
5. Norepinephrine infusion does not affect fetal acid-base balance.

We speculate that fetal hypertension has no effect on umbilical artery waveform indices in fetuses with normal placentation and that the fetus is capable of regulating placental perfusion pressure by altering systemic arterial pressure and umbilical venous tone.

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Inhibition of oxytocin-induced uterine contractions by an oxytocin antagonist in the pregnant baboon

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Uterine contractions were induced with oxytocin in anesthetized pregnant baboons *(Papio anubis)* at three stages of pregnancy (days 140, 156, and 169; normal gestation length, 184 days). After the contractile activity was greater than two to three contractions every 10 minutes, β-mercapto-β, β-cyclopentamethylenepropionic acid^1-[D-Trp^2,Phe^3,Ile^4,Arg^8]-oxytocin, a novel oxytocin antagonist produced in our laboratories, was given simultaneously with the oxytocin for 90 minutes. Contractile force (frequency × mean amplitude) was determined for 30 minutes before and for three 30-minute intervals after the oxytocin antagonist was administered. Animals at 140 days' gestation showed a significant ($p < 0.05$) decrease in contractile force in the first 30-minute interval after oxytocin antagonist infusion was initiated, whereas those at days 156 and 169 showed decreases ($p < 0.05$) at the second 30-minute interval. In addition, in late gestation a higher dose of oxytocin antagonist per unit of oxytocin was required to prevent uterine contractions. In conclusion, these results suggest (1) that an oxytocin antagonist can inhibit oxytocin-induced uterine contractions in the pregnant baboon and (2) that the interval from oxytocin antagonist administration to significant inhibition of uterine contractions appears to increase with advancing gestational age. (AM J OBSTET GYNECOL 1991;165:456-60.)

Key words: Oxytocin antagonist; baboon, pregnant; uterus, contractions; premature labor

Preterm labor and delivery is a major cause of perinatal mortality and morbidity.[1] Potential therapeutic modalities used to delay preterm labor include β-sympathomimetics, magnesium sulfate, calcium blockers, and indomethacin. These treatments can have side effects on the mother or fetus and often are not effective. An oxytocin antagonist has the potential to induce fewer side effects because of the limited number of target tissues for oxytocin. In addition, an oxytocin antagonist would help determine the contribution of endogenous oxytocin to uterine contractile activity during pregnancy and labor.

Recent reports have emphasized the clinical potential of an oxytocin antagonist.[2-6] We have reported the effects of a potent oxytocin antagonist produced in our laboratory that in the rat (1) inhibits uterine contractions induced by exogenous oxytocin in vitro and in vivo, (2) inhibits milk let-down, and (3) disrupts labor.[7] In addition, the oxytocin antagonist inhibited in vitro uterine contractions induced by oxytocin in myometrial tissue obtained at term pregnancy in humans.

The purpose of this study was to demonstrate that the oxytocin antagonist can inhibit uterine contractions induced by oxytocin in the pregnant baboon in vivo and thus establish the potential of the oxytocin antagonist as a tocolytic for preterm labor in humans.

Material and methods

Baboons *(Papio anubis)* with timed pregnancies from the breeding colony of the Biological Resource Laboratory at the University of Illinois were used in these studies. These animals are kept under controlled lighting conditions (12 hours light and 12 hours dark) with lights on at 6 AM and are given food (Purina Primate Chow [Ralston Purina, St. Louis], fruit, and nuts) and water as desired. Normal delivery occurs at 184 ± 2 days (day of mating, day 0). The protocols in this report have been approved by the Animal Care Committee at the University of Illinois.

Five animals each were studied at three stages of pregnancy: days 138 to 142, 153 to 157, and 168 to 172, with the mean for each stage equal to 140, 156, and 169, respectively. Two animals delivered before the third-stage (day 169) study was performed. On the day (8 AM) of a study the animal was sedated with intramuscular injections of ketamine (7.0 mg/kg) and maintained on a regimen of intravenous ketamine drip (10 mg/kg/hr) and intramuscular injections of diazepam (0.5 to 1.0 mg/kg), administered every 30 to 45 min-

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utes. The total length of the protocol was 6 to 7 hours. Animals had an initial ultrasonography to locate the placenta and pockets of amniotic fluid for subsequent catheter insertion. A 1 mm outer diameter polyethylene cannula (PE50; Clay Adams, Parsippany, N.J.) was placed transabdominally through an 18-gauge Angiocath catheter (Becton Dickinson Deseret Div., Sandy, Utah) into the amniotic fluid, for monitoring uterine contractions as reflected by intrauterine pressure changes. To induce contractions, oxytocin was infused intravenously beginning at 10 pmol/min (i.e., 5 mU/min) and increasing the rate by 10 pmol/min every 20 to 30 minutes until uterine contractions attained frequencies of two to three every 10 minutes. The oxytocin antagonist, β-mercapto-β,β-cyclopentamethylenepropionic acid^1-[D-Trp^2,Phe^3,Ile^4,Arg^8]-oxytocin (molecular weight, 1145 d), was then infused simultaneously with the oxytocin, beginning with a bolus injection of 3000 nmol and followed by a continuous intravenous infusion of 16 nmol/min oxytocin antagonist until all uterine contractions were suppressed. If uterine contractions remained after oxytocin antagonist treatment was initiated, subsequent bolus injections of oxytocin antagonist (3000 nmol) were given at 30-minute intervals.

Intrauterine pressure changes were monitored by an internal pressure transducer attached to an Epcom (Epcom Medical Systems, Hayward, Calif.) fetal monitor (model 315).

Data were analyzed by calculating the contractile force (Frequency × Mean amplitude) at four 30-minute intervals, one before and three after initiation of the oxytocin antagonist administration. Examination of the data for heterogeneity of variance by Box and Cox transformation analysis was significant; therefore all data underwent square root transformation before analysis. Data were analyzed by repeat analysis of variance with time effect nested within day effect, and, when significant, differences were detected by Duncan's multiple range test.[8] When contractile force was converted to percent of baseline, statistical significance ($p < 0.05$) was determined when the mean plus two times the standard deviation of a given 30-minute interval did not overlap with the mean baseline.

Results

There was no statistical difference ($p < 0.05$) among the maternal body weights (mean ± SEM) of 17.4 ± 0.9, 17.4 ± 0.8, and 18.2 ± 0.7 kg at days 140, 156, and 169 of pregnancy, respectively. The effects of oxytocin and oxytocin antagonist on uterine contractions, expressed as contractile force (Frequency × Mean amplitude) or as a percentage contractile force of the baseline, are illustrated in Fig. 1, *A* and *B*, respectively. An immediate inhibitory effect (first 30-minute interval) of the oxytocin antagonist on contractile force was detected ($p < 0.05$) at day 140 of pregnancy (Fig. 1, *A*), whereas animals at 156 and 169 days of pregnancy have a significant decrease from baseline ($p < 0.05$) at the second 30-minute interval after oxytocin antagonist administration. However, comparisons within the second 30-minute time interval revealed that activity on day 169 was higher ($p < 0.05$) than on days 140 and 156, but by the third 30-minute interval after oxytocin antagonist administration no differences ($p > 0.05$) were detected among the three stages of pregnancy studied. Thus the oxytocin antagonist was very effective in inhibiting uterine contractions induced by oxytocin, but the interval between administration and significant inhibition of uterine con-

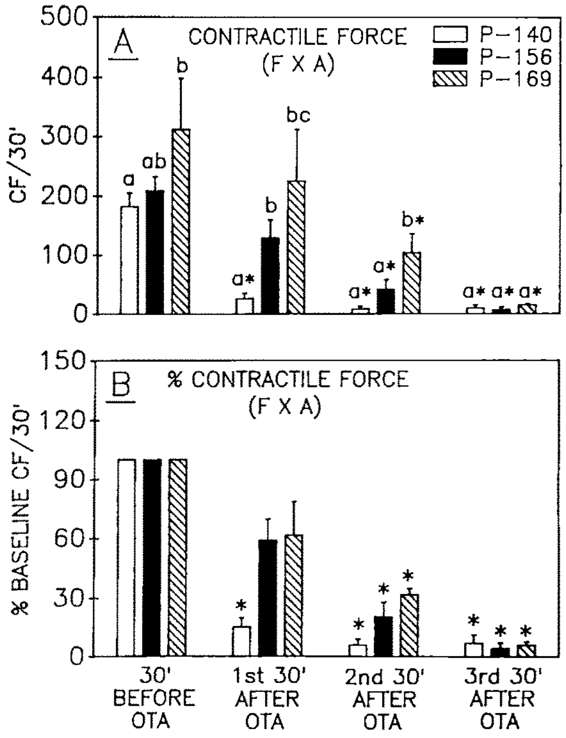

Fig. 1. Depicted are oxytocin-induced uterine contractile force (Frequency × Mean amplitude) (**A**) and percent contractile force *(CF)* of baseline (**B**) in pregnant baboons studied at three stages of pregnancy. Dose of oxytocin infused was incremented until two to three contractions occurred every 10 minutes; it then was infused at this dose for 30 minutes, followed by simultaneous administration of oxytocin antagonist *(OTA)* for 90 minutes. Data were analyzed at four intervals, one 30-minute interval before and three 30-minute intervals after infusion of oxytocin antagonist. Oxytocin antagonist inhibited uterine contractions induced by oxytocin, but the interval from administration of oxytocin antagonist to significant contractile force suppression increased with advancing gestational age. *Bars with different letters* within given 30-minute interval are significantly ($p < 0.05$) different from each other. *Asterisk* indicates differences ($p < 0.05$) from baseline control values ($n = 5$, 5, and 3 for days 140, 156, and 169, respectively; mean ± SEM). **B,** *Asterisk* indicates mean percent contractile force was greater than 2 SD (i.e., $p < 0.05$) from baseline.

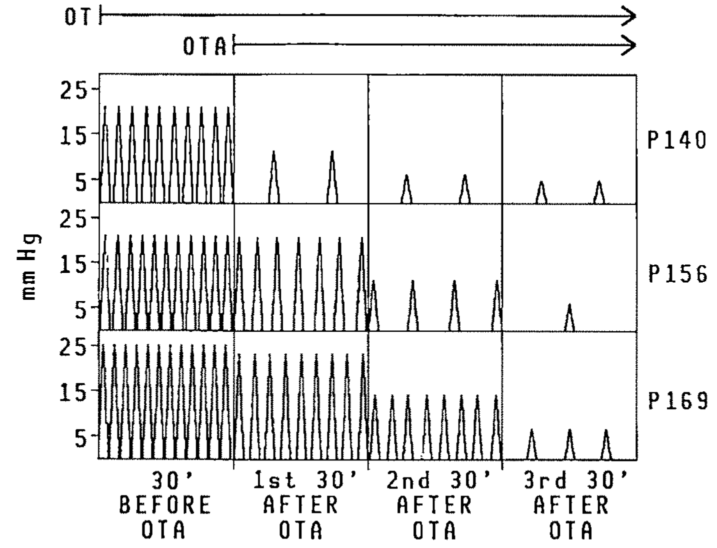

Fig. 2. Diagram showing same data as in Fig. 1 but representing mean frequencies and amplitudes during each 30-minute interval at three stages of pregnancy (days 140, 156, and 169) studied.

tractile force appeared to increase with advancing gestational age. In all instances a significant inhibition of uterine contractile force was noticed within 60 to 90 minutes after the oxytocin antagonist administration. The mean total dose of oxytocin antagonist administered over the 90-minute interval was 5680, 8136, and 7840 nmol for days 140, 156, and 169, respectively. Thus, on average, animals at days 156 and 169 received slightly less than one additional bolus injection of oxytocin antagonist, in addition to the initial bolus injection of 3000 nmol and subsequent oxytocin antagonist infusion. Furthermore, the total amount of oxytocin infused over 90 minutes at days 140, 156, and 169 was 0.405, 0.342, and 0.149 pmol; thus the ratio of oxytocin antagonist to oxytocin infused was about four times greater at day 169 than at day 140. Baseline uterine contractile force (30-minute interval before oxytocin antagonist) was greater on day 169 ($p < 0.05$) than on day 140, even though the trend was for a lower oxytocin infusion rate during this period (mean ± SEM, 45.0 ± 12.6, 38.0 ± 6.6, and 16.6 ± 6.6 pmol oxytocin per minute for days 140, 156, and 169, respectively). This suggests an increased sensitivity of the uterus to oxytocin at this stage of pregnancy (Fig. 1, *A*).

The results of expressing the contractile force as a percentage of the initial baseline are shown in Fig. 1, *B*. Presenting the data as a percentage of initial baseline adjusts for any incipient differences in contractile force and allows for a better appreciation of the relative uterine responses to the oxytocin antagonist at the different stages of pregnancy studied. Between 60 and 90 minutes after the oxytocin antagonist was given, all animals at the three stages of pregnancy studied showed a 95% reduction in contractile force as compared with initial baseline, even though oxytocin was administered continuously.

The frequency and amplitude of contractions are summarized diagrammatically in Fig. 2. The significance of this figure is that it represents the observed mean frequencies and amplitudes for each 30-minute interval and provides a visual comparison for the relative changes in activity of these two parameters.

Comment

This study demonstrates that the oxytocin antagonist, β-mercapto-β,β-cyclopentamethylenepropionic acid^1-[D-Trp^2,Phe^3,Ile^4,Arg^8]-oxytocin, produced in our laboratory inhibits uterine contractions induced by exogenous oxytocin in the pregnant baboon in vivo and that the interval from administration of the oxytocin antagonist to the time of significant inhibition of uterine contractile force appears to increase with advancing gestational age. These results suggest that the oxytocin antagonist has the potential of inhibiting preterm labor in humans, assuming endogenous oxytocin has a significant role in this process. Although the latter point has been controversial,[9-11] recent observations in our laboratory indicate that the oxytocin antagonist can disrupt labor in pregnant rats[7] and inhibit both spontaneous nocturnal and labor contractions in pregnant baboons,[12] thus adding support to this concept.[13] Similarly, Hahn et al.[3] have reported in pregnant rats a delay in delivery time of pups with the use of an oxytocin antagonist that is different from the one described here. In addition, the reports of Akerlund et al.[2] and Andersen et al.,[6] that a structurally different oxytocin antagonist administered to humans can stop preterm labor contractions, suggest that the oxytocin antagonist described in the present study has a similar potential.

We have concluded that the oxytocin antagonist is more effective in early than late stages of the last third of pregnancy on the basis of the observation that the interval required for the oxytocin antagonist to suppress uterine contractions increases with advancing gestational age. These results are even more impressive if one considers that the total dose of oxytocin antagonist administered in this study was greater at days 156 and 169 than at day 140, and yet the rapidity of effectiveness of the oxytocin antagonist was diminished at these later stages of pregnancy. These data correlate with the observation in our laboratory that the oxytocin antagonist is more effective in suppressing uterine contractions induced by exogenous oxytocin in an in vitro oxytocic bioassay in tissue obtained from day 15 of pregnancy in the rat compared with delivery (unpublished observations). An explanation for the decrease in effectiveness of the oxytocin antagonist with advancing gestational age is only speculative. It seems not to be a function of altered oxytocin antagonist metabolism or half-life, because it occurs in vitro with rat uterine tissue. However, an effect on oxytocin metabolism in vivo cannot be totally eliminated. Uterine oxytocin receptor number increases dramatically at the end of pregnancy in a number of species.[13-15] It is likely that our results are related to the probability of oxytocin and oxytocin antagonist interacting with the oxytocin receptor or to changes in postreceptor amplification of the signal. Finally, it is possible that decreased effectiveness of the oxytocin antagonist with advancing gestation is simply related to the higher contractile force baseline. However, this explanation seems unlikely for several reasons. First, and most relevant, is Fig. 1, *B*. The expression of the contractile force as a percentage of the baseline corrects for any initial baseline differences. The delayed effect of the oxytocin antagonist with advancing gestation is apparent even when this correction is made. Second , the baseline contractile force at day 156 did not differ ($p > 0.05$) from that at day 140, yet there was a statistically significant delay in oxytocin antagonist action at this stage of pregnancy. Further studies will be required to provide a definitive explanation.

The observation that the uterus appears to be more sensitive to oxytocin at day 169 than at day 140 is most likely related to an increase in oxytocin receptor number between days 140 and 169. Uterine oxytocin receptor number has been reported to increase with advancing gestational age in other species such as the rat and human,[13-15] and this likely occurs in the baboon uterus as well.

The effectiveness of the oxytocin antagonist in inhibiting uterine contractions in this study are very impressive, considering that the response was achieved while exogenous oxytocin was continuously infused. In normal pregnancies the release of endogenous oxytocin is pulsatile and responds reflexively (Ferguson reflex) to the positive feedback effects of uterine contractions and cervical stretching.[16,17] Thus one might expect the oxytocin antagonist to be more effective in blocking spontaneous uterine contractions induced by endogenous oxytocin, because the endogenous release of oxytocin is most likely decreased as the uterine contractions diminish. Therefore the oxytocin antagonist dosage and the length of administration required in this study are probably greater than those necessary to inhibit spontaneous uterine contractions induced by endogenous oxytocin. This assumption is supported by the observation by our laboratory that only a single bolus injection of 3000 nmol of oxytocin antagonist is required to inhibit both spontaneous nocturnal and labor uterine contractions in the pregnant baboon.[12]

Finally, although this part of the study is not shown, after the oxytocin antagonist infusion was stopped, the oxytocin infusion was continued, often at much higher levels (e.g., 60 pmol/min), to determine if uterine contractions might be reestablished. Uterine contractions usually began within 15 to 20 minutes, although not at the level of uterine activity detected before the oxytocin antagonist was administered. Time limitations prevented an adequate assessment of this observation. However, in a tethered baboon model developed in our laboratory, where spontaneous uterine contractions of labor were suppressed with several large doses of the oxytocin antagonist, infusion of oxytocin 1 hour after the termination of the oxytocin antagonist administration prompted immediate uterine contractions that ultimately resulted in labor 3½ hours later.[12] Therefore it seems likely that after uterine contractions are suppressed with the oxytocin antagonist, this effect can be reversed with oxytocin within 1 hour, if not less. In addition, if necessary, prostaglandin F_{2a} or E_2 can be administered because each can induce uterine contractions in the presence of the oxytocin antagonist in the rat[7] and the pregnant baboon (unpublished observations).

In conclusion, a potent oxytocin antagonist has been developed in our laboratory that inhibits in vivo uterine contractions induced by exogenous oxytocin in the pregnant baboon. The interval required for inhibition of uterine contractions appears to increase with advancing gestational age, but the oxytocin antagonist ultimately inhibited uterine contractions in all studies within 60 minutes after the initiation of oxytocin antagonist administration. The results of these and other studies suggest that the oxytocin antagonist has the potential of inhibiting labor, and probably preterm labor, contractions in humans.

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The prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the families of couples with recurrent spontaneous abortions or gestational trophoblastic tumors

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This study extends our previous work on the genetics of recurrent spontaneous abortion and of gestational trophoblastic tumors in an ethnically homogeneous population of Chinese in Taiwan by comparing the prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the first-, second-, and third-degree relatives of the index couples to that of normally fertile couples from the same population. The rationale for this study was to provide another test for our hypothesis that genes linked to the major histocompatibility complex are responsible for the diseases in the index couples. If they are, these genes should segregate with a higher frequency in the relatives of the index couples than in the relatives of normally fertile couples and lead to a higher prevalence of these diseases in the extended families. Such a difference was found and adds support to our hypothesis that major histocompatibility complex–linked genes affect growth, development, and susceptibility to cancer. (AM J OBSTET GYNECOL 1991;165:461-6.)

Key words: Genetics of reproduction, genetics of cancer, human leukocyte antigen sharing, recurrent spontaneous abortion, congenital anomalies

Experimental studies in rats and observations in the clinical literature led us to develop the hypothesis that recessive genes linked to the major histocompatibility complex influence growth, development, reproduction, and susceptibility to cancer.[1-5] We tested this hypothesis in a series of clinical studies in an ethnically homogeneous Chinese population in Taiwan (originally emigrated from the southeastern part of mainland China) to minimize the genetic variation in the population. The basic assumptions of the studies were that sharing of major histocompatibility complex antigens [human leukocyte antigens (HLA) in human beings] indicates the sharing of the major histocompatibility complex–linked region as well and that this region contains recessive genes that affect the growth and reproductive processes. We examined couples for HLA antigen sharing and the prevelance of gestational trophoblastic tumors and of recurrent spontaneous abortions and compared the results with those in normally fertile couples from the same population.

Couples in whom the woman had a gestational trophoblastic tumor[6] and couples with primary or secondary recurrent spontaneous abortions[7] shared HLA antigens more frequently than did normally fertile couples. No specific HLA antigen was uniquely shared, and the strongest correlation was sharing of three or more of the HLA-A, B, DR, and DQ antigens. This observation strengthens our hypothesis that the HLA genes themselves are only passenger genes and that the genes responsible for these defects are on the same segment of chromosome 6 and linked to the genes encoding the HLA antigens. Two collateral observations are consistent with this genetic interpretation. First, gestational trophoblastic tumors have a high prevalance in the Taiwan Chinese population and a very low prevalence in whites, and the genetic association was observed only in the Chinese population in Taiwan: thus the disease is genetically determined (familial) in Taiwan Chinese and sporadic in whites. Retinoblastoma also has familial and sporadic forms.[8, 9] Second, immunotherapy for recurrent spontaneous abortion was not effective in this Taiwanese population, as would be expected for a genetically determined disease.[10]

This study extends our work in this Taiwan Chinese population to examine the prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the first-, second- and third-degree relatives of the index couples (i.e., those couples with gestational trophoblastic tumors or recurrent spontaneous abortions in our previous studies).[6, 7] The rationale for this

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Table I. The prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the first-degree relatives of couples who are normally fertile, have recurrent spontaneous abortions, or have gestational trophoblastic tumors

	Reproductive histories of the index couples				
	Normally fertile (controls)	*Recurrent abortions*			*Gestational trophoblastic tumors*
Population		*Primary*	*Secondary*	*Total*	
No. of index couples whose families were studied	406	158	60	218	22
First-degree relatives					
Number	2519	632	302	934	113
Recurrent spontaneous abortions	4	9	4	13	1
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	NS
Cancer					
Total	95	45	25	70	8
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.03$
<40 years old*	7	6	2	8	0
		$p < 0.02$	NS	$p < 0.02$	
Congenital anomalies	18	1	4	5	1
		NS	NS	NS	NS

*Cancers occurring at a young age (e.g., childhood leukemia) were not included.

Table II. The prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the second- and third-degree relatives of couples who are normally fertile, have recurrent spontaneous abortions, or have gestational trophoblastic tumors

	Reproductive histories of the index couples				
	Normally fertile (controls)	*Recurrent abortions*			*Gestational trophoblastic tumors*
Population		*Primary*	*Secondary*	*Total*	
Second-degree relatives					
Number	3536	1306	513	1819	170
Recurrent spontaneous abortions	11	12	10	22	4
		$p < 0.009$	$p < 0.0001$	$p < 0.005$	$p < 0.0001$
Cancer					
Total	29	33	22	55	3
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	NS
<40 years old*	6	6	7	13	0
		NS	$p < 0.02$	$p < 0.001$	
Congenital anomalies	8	14	2	16	2
		$p < 0.0001$	NS	$p < 0.008$	$p < 0.002$
Third-degree relatives					
Number	11603	4367	1601	5968	554
Recurrent spontaneous abortions	3	11	1	12	2
		$p < 0.0001$	NS	$p < 0.0001$	$p < 0.0001$
Cancer					
Total	99	34	23	57	9
		NS	$p < 0.02$	NS	NS
<40 years old*	12	2	5	7	1
		NS	$p < 0.02$	NS	NS
Congenital anomalies	29	17	16	33	5
		NS	$p < 0.0001$	$p < 0.005$	$p < 0.003$

*Cancers occurring at a young age (e.g., childhood leukemia) were not included.

approach to test our hypothesis was that the major histocompatibility complex–linked genes responsible for the diseases in the index couples should segregate with a higher frequency in their relatives than in the relatives of the normally fertile couples and lead to a higher prevelance of recurrent spontaneous abortions, cancer, and congenital anomalies in the relatives of the index couples. Such a relationship was found and, coupled with our previous studies,[6,7] adds further support to our hypothesis that major histocompatibility complex–linked genes affect growth, development, and susceptibility to cancer. These major histocompatibility complex–linked genes may be related to tumor suppressor genes,[8,9] and such genes may play a broad role

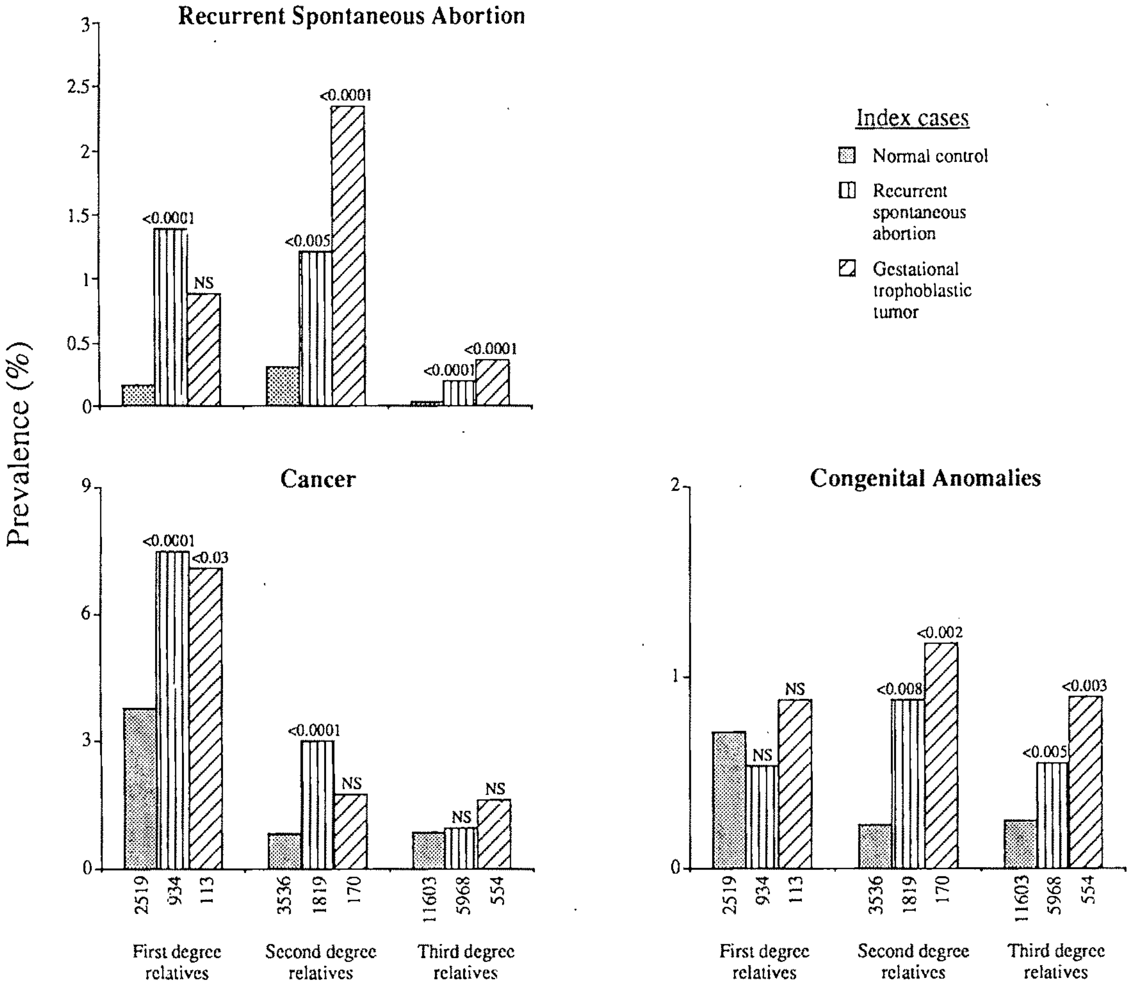

Fig. 1. Prevalence of recurrent spontaneous abortion, cancer, and congenital anomalies in the first-, second-, and third-degree relatives of couples who were normally fertile, who experienced recurrent spontaneous abortions, or in whom a gestational trophoblastic tumor developed in the woman.

in the control of growth and not be confined only to a role in oncogenesis.

Material and methods

The couples whose families were examined (index couples) were those who had previously been studied for gestational trophoblastic tumors, recurrent spontaneous abortions, and the effects of immunotherapy. They were all patients from the Obstetrics Clinic of the National Taiwan University Hospital, and they have been described in detail.[6, 7, 10]

The first-, second- and third-degree relatives of both the husband and the wife in the index couples and in the normally fertile controls were carefully interviewed by two nurses who were well trained in interviewing techniques and who were unaware of the purpose of the interview. The interview focused on a complete reproductive history, with special emphasis on recurrent (at least three) spontaneous abortions, and on the presence and nature of cancer and of congenital anomalies in any living or dead relative. The data on cancer were collected as the total prevalence and as the prevalence in persons younger than 40 years old exclusive of childhood cancers such as leukemia (childhood data would not be reliable in this population). After the interview, a questionnaire covering the same information was sent to those interviewed to cross-check the information. If there was any discrepancy, a second interview was scheduled to reconcile the differences.

The data from the families of the index couples were compared with those of the families of normally fertile couples with the use of a 2×2 contingency test; $p \leq 0.05$ was the level of significance.

Results

The prevalence rates of recurrent spontaneous abortions, cancer, and congenital anomalies for the first-degree relatives of the index and control couples are

Table III. The distribution of cancers among the families of normally fertile couples, those with primary and secondary recurrent spontaneous abortions, and women with gestational trophoblastic tumors

	Type of patient whose family was studied			
	Normally fertile (controls)	*Recurrent abortions*		*Gestational trophoblastic tumors*
		Primary	*Secondary*	
First-degree relatives				
Hepatoma	25	10	2	—
Cervix	14	7	5	3
Stomach	13	4	5	—
Lung	7	6	3	1
Colon	4	4	2	—
Nasopharynx	4	1	2	—
Breast	4	3	—	—
Leukemia	1	3	1	2
Lymphoma	3	—	1	2
Pancreas	5	1	—	—
Bone	1	1	—	—
Esophagus	3	2	—	—
Brain	1	—	—	—
Bladder	1	—	—	—
Penis	—	1	—	—
Unknown origin	9	2	4	—
Second-degree relatives				
Hepatoma	5	6	3	1
Cervix	2	9	3	—
Stomach	2	1	4	—
Lung	4	2	2	—
Colon	2	4	2	—
Nasopharynx	3	2	1	—
Breast	1	2	2	—
Leukemia	3	2	—	—
Lymphoma	—	—	1	1
Pancreas	—	2	1	—
Skin	1	1	—	—
Esophagus	—	1	—	—
Kidney	1	—	—	—
Larynx	1	—	—	—
Mouth	1	—	—	—
Ovary	—	—	1	—
Unknown origin	3	1	2	—
Third-degree relatives				
Hepatoma	20	2	5	1
Cervix	7	4	4	2
Stomach	12	3	6	—
Lung	14	3	2	—
Colon	7	1	2	—
Nasopharynx	8	2	1	—
Breast	6	4	3	1
Leukemia	3	5	—	1
Lymphoma	3	—	—	1
Pancreas	3	—	—	—
Bone	3	2	—	—
Brain	1	—	—	2
Larynx	—	1	—	—
Bladder	—	1	—	—
Esophagus	1	1	—	—
Unknown origin	11	5	—	1

given in Table I; data for the second- and third-degree relatives are in Table II. The data are summarized graphically in Fig. 1; for recurrent spontaneous abortions, only the total prevalence is shown. When either couples with recurrent spontaneous abortions or couples in whom a gestational trophoblastic tumor developed in the woman are taken as the index cases, there is a higher prevalence of recurrent spontaneous abortions, cancer, and congenital anomalies in the first-, second-, and third-degree relatives. The only significant exceptions to this generalization are the lack of cancer in the third-degree relatives and the lack of congenital anomalies in the first-degree relatives. There is no clear reason why the correlation with cancer should

Table IV. The distribution of congenital anomalies among the families of normally fertile couples, those with primary and secondary recurrent spontaneous abortions, and women with gestational trophoblastic tumors

	Type of patient whose family was studied			
	Normally fertile (controls)	*Recurrent abortions*		*Gestational trophoblastic tumors*
		Primary	*Secondary*	
First-degree relatives				
Chromosomal abnormalities	2	—	—	—
Congenital heart disease	2	1	1	—
Cleft lip or palate	2	—	1	—
Polydactyly	2	—	—	—
Hernia	2	—	—	—
Enzyme defect	—	—	2	—
Renal cyst	—	—	—	1
Multiple defects	8	—	—	—
Second-degree relatives				
Chromosomal abnormalities	1	2	—	—
Congenital heart disease	2	5	—	1
Cleft lip or palate	2	—	—	1
Polydactyly	1	1	1	—
Hernia	1	6	—	—
Multiple defects	1	1	1	—
Third-degree relatives				
Chromosomal abnormalities	2	2	—	—
Congenital heart disease	4	4	1	2
Cleft lip or palate	8	3	2	—
Polydactyly	2	1	2	2
Albinism	1	—	3	—
Hernia	3	5	1	—
Torticollis	—	—	2	—
Esophageal atresia	—	1	—	—
Anencephaly	—	—	—	1
Multiple defects	9	—	5	—

be lost in the third-degree relatives unless some of the genes involved in the susceptibility to cancer were lost in the more distant relatives. Such diffusion of genes in a population has been described by Cavalli-Sforza and Bodmer.[11] The number of first-degree relatives examined may be too small as compared with the prevalence of congenital anomalies in the population to show a significant deviation from the population mean. This explanation appears reasonable because of the significant increase in congenital anomalies in the second- and third-degree relatives for whom the numbers examined are greater.

The distribution of cancers (Table III) and of congenital anomalies (Table IV) in the first-, second-, and third-degree relatives of the normally fertile controls and of the index cases is quite broad and does not show a pattern unique to any one group. The relatively high prevalence of hepatoma in all groups reflects the high prevalence of this disease in the Taiwan Chinese population.[12, 13]

Comment

The evidence of the increase in recurrent spontaneous abortions, cancer, and congenital anomalies in the first-, second-, and third-degree relatives of couples with recurrent spontaneous abortions or of couples in whom a gestational trophoblastic tumor developed in the woman provides epidemiologic support for our hypothesis that major histocompatibility complex–linked genes are involved in the pathogenesis of these diseases. The data from this and other clinical studies[6, 7] and from experimental studies[4, 5, 14] provide strong enough support for this hypothesis to warrant studies at the molecular level to identify the major histocompatibility complex–linked genes involved.

An interesting speculation may be made on the basis of the clinical data presented here and of experimental evidence in rats. Studies in rats have shown that there are at least two separate major histocompatibility complex–linked genes or groups of closely linked genes that affect growth and reproduction: *dw-3*, which affects body size, and *ft*, which affects fertility.[14] Loss of these genes leads to growth defects, partial loss (25% to 30%) of homozygous offspring, and increased susceptibility to chemical carcinogens.[15] Possibly the phenotypes of primary or secondary recurrent spontaneous abortion, increased susceptibility to cancer, and congenital anomalies are a result of losses of different constellations of similar genes in human beings. The severity of a particular defect, measured roughly by the length of survival of the person carrying it, may reflect the number of these genes lost: embryonic death more than primary recurrent spontaneous abortion more than secondary recurrent spontaneous abortion more than susceptibility to cancer. Whereas this explanation may eventually prove to be too simple, it provides one framework within which to examine the genetics of growth and reproduction, and it is consistent with the concept of tumor suppressor genes and the increased susceptibility to cancer after their loss.[8, 9]

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The mechanism responsible for diminished neutrophil production in neonates delivered of women with pregnancy-induced hypertension

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The neonatal neutropenia after pregnancy-induced hypertension is a function of diminished neutrophil production. These studies test the hypothesis that this diminution is due to decreased production of neutrophilic growth factors, reduced responsiveness of neutrophil progenitors to these factors, or the presence of an inhibitor. While the concentrations of placentally derived colony-stimulating factors were similar in normotensive and hypertensive gestations, bioassay demonstrated less colony-stimulating activity in placental conditioned media from hypertensive gestations. Evaluation of the responsiveness of progenitors to recombinant factors revealed no differences between those from normotensive and hypertensive gestations. However, neutrophilic colony formation in vitro was significantly inhibited after the addition of conditioned media or sera from hypertensive gestations, whereas the addition of these from normotensive gestations had no inhibitory effect. Thus this common maternal-fetal disorder is associated with an inhibitor of neutrophil production, which is elaborated by the placenta and present in cord blood serum. (AM J OBSTET GYNECOL 1991;165:467-73.)

Key words: Neonate, neutrophil, pregnancy-induced hypertension, inhibitor

Neutropenia is frequently observed in neonates delivered of women with pregnancy-induced hypertension.[1-3] Studies by Manroe et al.,[1] Brazy et al.,[2] Engle and Rosenfeld,[3] and our group[4] indicate that neutropenia is present in 40% to 50% of such neonates and in up to 80% of neonates when maternal hypertension is severe (diastolic blood pressure ≥110 mm Hg). Although neutropenia is usually transient, these neonates may be at greater risk for nosocomial infection in the first weeks of life.[4]

Our previous investigations have established that the neonatal neutropenia associated with pregnancy-induced hypertension is the kinetic result of diminished neutrophil production.[4] We postulated that determination of the mechanism resulting in this diminished production might facilitate our management of this neutropenia and further our understanding of this common maternal-fetal disorder. These studies were performed to test the hypothesis that the cause of the reduced neutrophil production might be one of the following: (1) diminished production of the neutrophilic growth factors, granulocyte colony-stimulating factor, or granulocyte-macrophage colony-stimulating factor; (2) reduced responsiveness of neutrophil progenitor cells to granulocyte or granulocyte-macrophage colony-stimulating factors; or (3) elaboration of an inhibitor of fetal neutrophil production.

Material and methods

Subjects. Study subjects included six neonates (range, 28 to 34 weeks) delivered of mothers with severe pregnancy-induced hypertension (diastolic pressure ≥110 mm Hg on two readings ≥6 hours apart[5]) and seven gestational age–matched neonates (range, 24 to 37 weeks) delivered after normotensive, uncomplicated gestations. None of the neonates delivered of hypertensive women were neutropenic for >30 hours.

To provide target cells for the clonogenic assays, normal hematopoietic progenitor cells were obtained from marrow aspirates of seven healthy adult volunteers. All studies were performed according to a protocol approved by the University of Utah Institutional Review Board.

Serum concentrations and placental production of granulocyte and granulocyte-macrophage colony-stimulating factors. For the serum studies, cord blood was aseptically obtained immediately after delivery, and the resulting serum was stored at −70° C until assay.

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Serum concentrations of immunoreactive granulocyte and granulocyte-macrophage colony-stimulating factors were determined by enzyme-linked immunosorbent assay (ELISA, G-CSF, AmGen Laboratories, Thousand Oaks, Calif.; GM-CSF, Genzyme Corp., Boston). Colony-stimulating factor biologic activity in the serum was assessed by coculturing serum (or α-minimum essential medium, HyClone Laboratories, Logan, Utah, as a control) with normal hematopoietic progenitor cells in the absence of recombinant growth factors. As a positive control, recombinant granulocyte and granulocyte-macrophage colony-stimulating factors (Immunex Corporation, Seattle) were added to one set of cultures.

In other studies we evaluated production of these factors by placental tissue. Placentas were obtained immediately after delivery from hypertensive or normotensive gestations. With a sterile technique, the overlying membranes were stripped, and tissue from the fetal side of each placenta was minced and serially rinsed in α-minimum essential medium. Three pieces, with a total weight of 0.15 gm, were placed into 35 mm culture dishes (Becton Dickinson Labware, Oxnard, Calif.) containing 3 ml of α-minimum essential medium with 1% Nutradoma NS (Boehringer-Mannheim Corp., Indianapolis). Plates were then incubated at 37° C in a humidified 5% carbon dioxide–air mixture and supernatants were harvested after 48 hours. After centrifugation at 2000 *g* for 10 minutes, supernatants were filtered through a 0.22 μm filter (Millipore Products Division, Bedford, Mass.), and the filtrate was stored at −70° C until assay. Immunoreactive levels of the factors were determined in the placental conditioned media with an enzyme-linked immunosorbent assay, and colony-stimulating factor biologic activity was determined with our clonogenic assay, noted above.

Responsiveness of neutrophil progenitors to recombinant granulocyte and granulocyte-macrophage colony-stimulating factors. Samples of umbilical cord blood were obtained from the placental vein immediately after delivery and anticoagulated with 10 to 30 units of preservative-free heparin (Squibb-Marsam, Inc., Cherry Hill, N.J.) per milliliter of blood. After Ficoll-Hypaque (Pharmacia Inc., Piscataway, N.J.) centrifugation and one wash in α-minimum essential medium, the light-density (specific gravity, <1.077) mononuclear cells were depleted of T-lymphocytes and macrophages by means of plastic adherence and negative panning techniques, as previously described.[6] Cultures of neutrophil progenitor cells were performed with a modification[7] of the method of Iscove et al.[8] Cultures were plated in quadruplicate at cell concentrations of 0.05 to 0.20 × 10^6 cells per milliliter. After 10 days' incubation, granulocyte-macrophage colonies were enumerated in situ with an inverted stereomicroscope. In the studies that assessed sensitivity of fetal neutrophil progenitor cells to recombinant growth factors, either granulocyte or granulocyte-macrophage colony-stimulating factors were added to cultures at various concentrations (0.001, 0.01, 0.1, 1.0, or 2.0 ng/ml). In other studies recombinant factors (granulocyte and granulocyte-macrophage colony-stimulating factors, interleukin-3, and macrophage colony-stimulating factor [Immunex]) were added at a concentration of 1 ng/ml to the culture medium.

Experiments to detect inhibitors of neutrophil production. Bone marrow progenitor cells from healthy adult volunteers were cultured in the presence of maximally stimulatory (plateau) concentrations (1 ng/ml each) of several recombinant factors (granulocyte, macrophage, and granulocyte-macrophage colony-stimulating factors; interleukin-3). To these cultures was added cord blood serum from normotensive or hypertensive gestations (5% or 15% concentrations) or placental conditioned media from normotensive or hypertensive gestations (5% or 10% concentrations).

Erythropoietin concentrations in cord blood serum and in placental conditioned media were measured with an enzyme-linked immunosorbent assay (AmGen).

Statistical analysis. Statistical analysis was accomplished by either Student's *t* test, Mann-Whitney U test, or the Student–Newman-Keuls test for multiple comparisons, as applicable.

Results

Placental production and serum concentrations of granulocyte and granulocyte-macrophage colony-stimulating factors. The first studies examined placental elaboration and cord blood concentrations of granulocyte and granulocyte-macrophage colony-stimulating factors from both normotensive and hypertensive gestations. Elaboration of these factors from placentas of normotensive and hypertensive gestations did not differ, as determined by measuring immunoreactive concentrations in placental conditioned media (granulocyte colony-stimulating factor: 440 ± 210 pg/ml in normotensive gestations vs 480 ± 150 pg/ml in pregnancy-induced hypertension; granulocyte-macrophage colony-stimulating factor: 7 ± 4 pg/ml in normotensive gestations vs 12 ± 1 pg/ml in pregnancy-induced hypertension). However, bioassay demonstrated significantly less biologically active colony-stimulating factor in the conditioned media from hypertensive gestations (Fig. 1).

Immunoreactive granulocyte and granulocyte-macrophage colony-stimulating factor concentrations in cord blood from normotensive and hypertensive gestations were immeasurably low (≤5 pg/ml). Colony-stimulating factor bioassay revealed significantly less colony-stimulating factor in serum from hypertensive

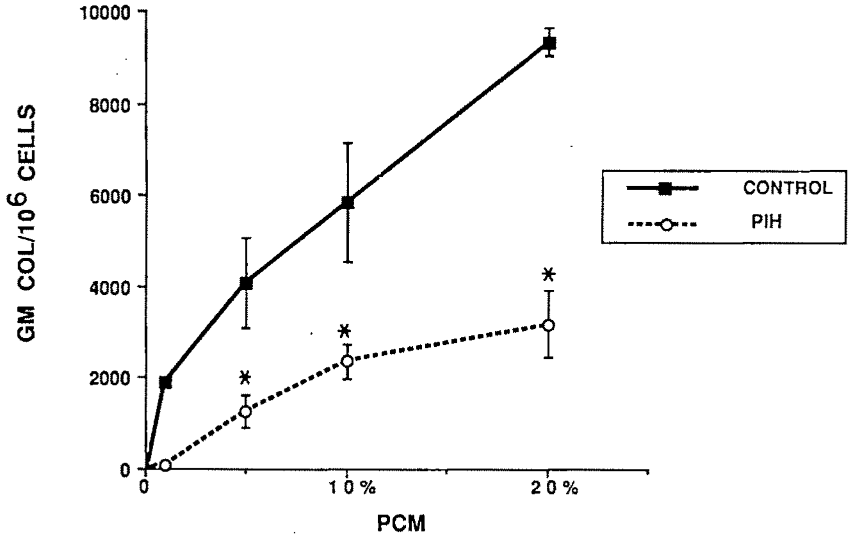

Fig. 1. Colony-stimulating factor biologic activity in placental conditioned media *(PCM)* from normotensive and hypertensive gestations. Data are expressed as mean ± SEM. *Control,* Normotensive ($n = 6$); *PIH,* pregnancy-induced hypertension ($n = 4$); *GM,* granulocyte-macrophage. *Asterisk,* $p < 0.001$, versus normotensive.

Table I. Granulocyte-macrophage colony formation in vitro after stimulation of cord blood progenitors with granulocyte or granulocyte-macrophage colony-stimulating factors

	Granulocyte-macrophage colonies (No. of colonies/10^6 cells plated)	
	Control ($n = 7$)	*Pregnancy-induced hypertension ($n = 6$)*
Granulocyte colony-stimulating factor	3050 ± 454	387 ± 90*
Granulocyte-macrophage colony-stimulating factor	5180 ± 740	650 ± 170*

Numbers in parentheses denote number of subjects. Data are expressed as mean ± SEM.
*$p < 0.001$, versus control.

gestations (7 ± 4 colonies per 10^6 plated cells vs 44 ± 17 colonies per 10^6 plated cells in normotensive gestations, $p < 0.05$). In addition, sera from both groups yielded fewer granulocyte-macrophage colonies than when the cultures were stimulated with 1 ng/ml each of granulocyte and granulocyte-macrophage colony-stimulating factor (4497 ± 75 colonies per 10^6 plated cells, $p < 0.001$).

Responsiveness of neutrophil progenitors to recombinant granulocyte and granulocyte-macrophage colony-stimulating factor. We first examined cord blood concentrations of neutrophil progenitor cells (±SEM) after hypertensive and normotensive gestations when stimulated with plateau concentrations of granulocyte (2 ng/ml) or granulocyte-macrophage colony-stimulating factor (1 ng/ml). As shown in Table I, relatively few neutrophil progenitor cells were detected in cord blood after hypertensive gestations. We next evaluated the in vitro sensitivity of fetal neutrophil progenitor cells from hypertensive and normotensive gestations to recombinant granulocyte and granulocyte-macrophage colony-stimulating factor (Fig. 2). As shown in the figure, there were no differences observed between the normotensive (control) and hypertensive (pregnancy-induced hypertension) gestations with respect to the responsiveness of their progenitors to granulocyte (upper panel) or granulocyte-macrophage colony-stimulating factor (lower panel).

Experiments to detect inhibitors of neutrophil production. We evaluated placental conditioned media and cord blood for the presence of an inhibitor of neutrophil production. As shown in Fig. 3, granulocyte-macrophage colony growth in the presence of recombinant factors alone (control group) was similar to that observed when the recombinant factors were combined with placental conditioned media from normotensive gestations. However, granulocyte-macrophage colony growth was significantly inhibited when placental con-

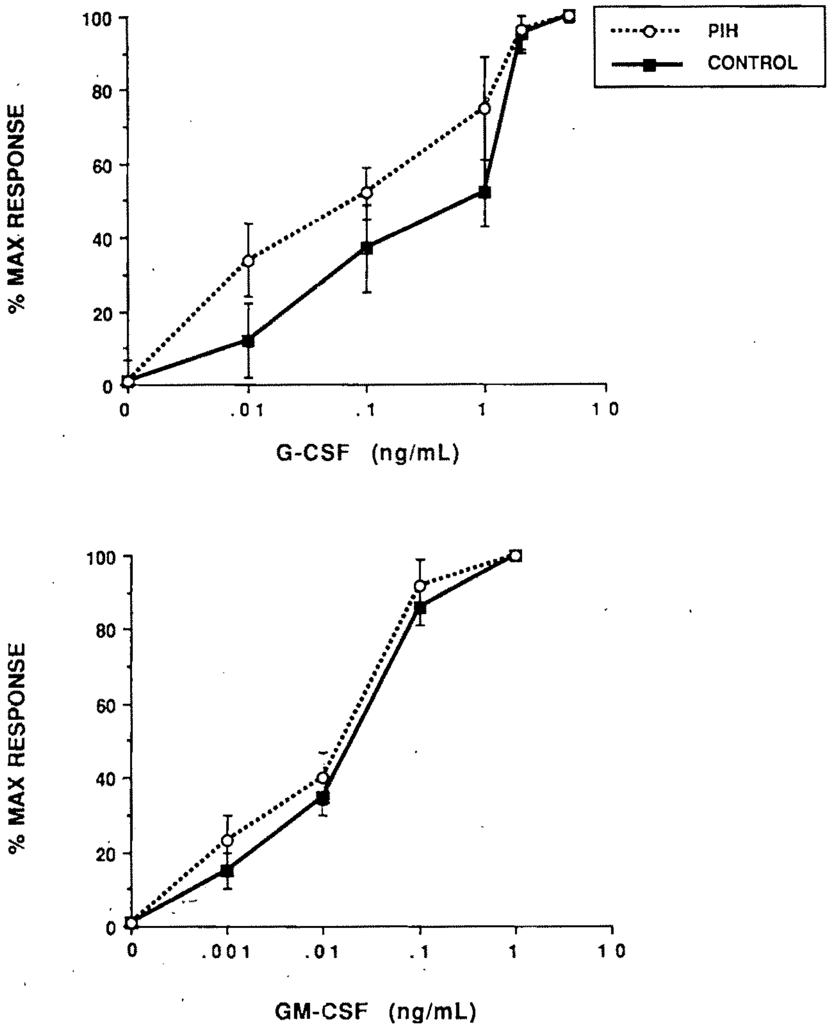

Fig. 2. Percentage of clonogenic response observed at maximal (plateau) concentrations of granulocyte colony-stimulating factor *(G-CSF)* or granulocyte-macrophage colony-stimulating factor *(GM-CSF)*. Data are expressed as mean ± SEM. *Control,* Normotensive ($n = 7$); *PIH,* pregnancy-induced hypertension ($n = 6$). *Asterisk,* $p < 0.01$, versus normotensive.

ditioned media from hypertensive gestations were added to the cultures. Further inhibition was noted when the concentration of the placental conditioned media was increased to 10%. In other studies sera from neonates delivered of normotensive or hypertensive women were added to cultures containing recombinant factors (Fig. 4). As with the placental conditioned media studies, granulocyte-macrophage colony growth was similar in cultures stimulated with the recombinant factors alone or in the presence of sera from normotensive gestations. Again, however, clonogenesis was significantly inhibited by sera from the hypertensive gestations.

Erythropoietin concentrations in the cord blood of neonates delivered of women with severe pregnancy-induced hypertension were significantly higher than in neonates with Rh isoimmunization, normal term infants, and otherwise healthy preterm neonates (Fig. 5). However, erythropoietin concentrations in the placental conditioned media of hypertensive and normotensive groups were similar (10.0 ± 0.2 vs 10.7 ± 0.3 mU/ml).

Comment

Neutropenia occurs in nearly half of all neonates delivered of women with pregnancy-induced hypertension.[1-4] Since hypertension complicates 5% to 6% of all pregnancies,[5] this variety of neutropenia occurs in 2% to 3% of all births, and therefore is the most common neutropenia during the first days of life. In previous studies we observed that the neonatal neutropenia associated with pregnancy-induced hypertension is the kinetic result of reduced fetal production of neutrophils.[4]

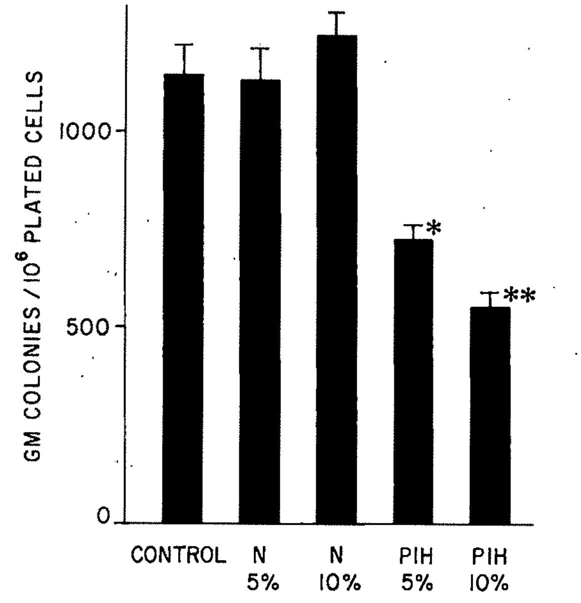

Fig. 3. Effect of placental conditioned media on granulocyte-macrophage *(GM)* colony formation. *N,* Normotensive ($n = 3$); *PIH,* pregnancy-induced hypertension ($n = 4$). Data are expressed as mean ± SEM. *Asterisk,* $p < 0.001$, versus *control* (normotensive); *two asterisks,* $p < 0.01$, versus 5% placental conditioned media from pregnancy-induced hypertension gestations.

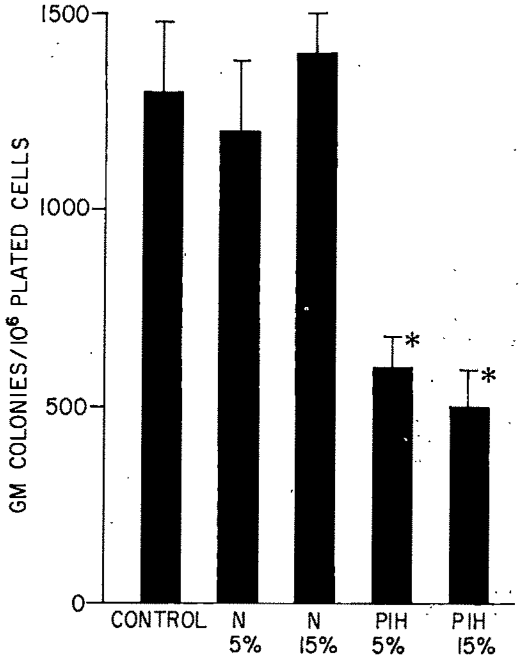

Fig. 4. Effect of umbilical venous serum on granulocyte-macrophage *(GM)* colony formation. *N,* Normotensive ($n = 5$); *PIH,* pregnancy-induced hypertension ($n = 5$). Data are expressed as mean ± SEM. *Asterisk,* $p < 0.001$, versus *control* (normotensive).

Neutrophil production occurs by clonal maturation of progenitor cells under the regulatory control of a family of glycoproteins known as hematopoietic growth factors.[9] Although the actions of several such factors appear to be required for neutrophil production in vitro,[9] studies in animals[10] and humans[11] indicate that granulocyte and granulocyte-macrophage colony-stimulating factors are particularly important. However, whereas interleukins IL-6 and IL-3 appear to act on primitive progenitors, synergism with the more lineage-specific granulocyte and granulocyte-macrophage colony-stimulating factors does occur.[9]

We hypothesized that the reduced neutrophil production might be the result of one, or a combination, of the following: (1) diminished production of granulocyte or granulocyte-macrophage colony-stimulating factors, (2) reduced responsiveness of neutrophil progenitor cells to granulocyte or granulocyte-macrophage colony-stimulating factors, or (3) elaboration of an inhibitor of neutrophil production. These studies were undertaken to examine each of these possibilities, so that additional investigations could concentrate on the most likely prospects.

In this study we did not observe any differences in elaboration of granulocyte and granulocyte-macrophage colony-stimulating factors by placental tissue obtained from hypertensive versus normotensive gestations. Studies by Burgess et al.,[12] Azoulay et al.,[13] and Bartocci et al.[14] illustrate the importance of the placenta in the generation of hematopoietic regulatory factors. It is clear that other tissues contribute an equal or greater role in their production. For example, endothelial cells,[15] fibroblasts,[16] macrophages,[17] and T lymphocytes[18] all display the capacity to elaborate granulocyte and granulocyte-macrophage colony-stimulating factors messenger ribonucleic acid transcripts and active granulocyte and granulocyte-macrophage colony-stimulating factors glycoproteins. Indeed, to conclude that the hyporegenerative neutropenia in neonates delivered of women with pregnancy-induced hypertension is the result of reduced fetal production of the neutrophilic growth factors, examination of production rates by all of these cell types in vivo would be required. Thus confirmation of our speculation that production of granulocyte and granulocyte-macrophage colony-stimulating factors is normal in these fetuses awaits further study. Similarly, although we have identified an in vitro inhibitor of neutrophil production elaborated by the placenta and present in cord blood in hypertensive gestations, we have not established conclusively its role as an inhibitor in vivo. In addition, diminished concentrations of unmeasured growth factors that are synergistic with granulocyte and granulocyte-macrophage colony-stimulating factors could also account for a reduction in biologic activity. In spite of these limitations, the consistencies in our

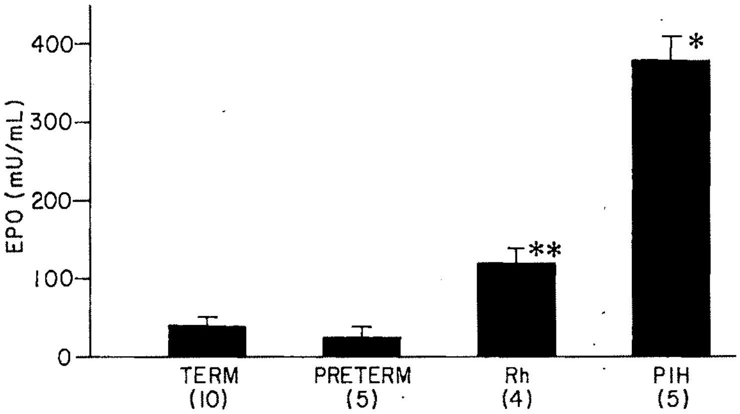

Fig. 5. Umbilical venous serum erythropoietin *(EPO)* concentrations. Numbers in parentheses are number of subjects. Data are expressed as mean ± SEM. *PIH,* Pregnancy-induced hypertension; *asterisk,* $p < 0.001$, versus term, preterm, and Rh neonates; *two asterisks,* $p < 0.001$, versus pregnancy-induced hypertension.

experimental results justify our speculation that the hyporegenerative neutropenia might be due to elaboration of an inhibitor of neutrophil production. None of the subjects in this study had the less common prolonged (>60 hours' duration) variety of neutropenia.[4] The mechanism that results in prolonged neutropenia and how this might differ from our observations would be an important topic for future investigation.

High concentrations of erythropoietin inhibit neutrophil production from progenitors in vitro,[19] and a growing body of evidence suggests that high erythropoietin concentrations also might inhibit fetal production of neutrophils in vivo. Specifically, neutrophil production is reduced in newborn rats injected with high concentrations of erythropoietin,[20] human fetuses exposed to high concentrations in utero generally have a hyporegenerative neutropenia during the first days of life,[21] and anemic neonates treated with recombinant erythropoietin can have neutropenia.[22] Because of these actions of erythropoietin on neutrophil production, we speculated that it might indeed be the inhibitor of neutrophil production elaborated by placental cells and present in the cord blood of fetuses delivered to women with pregnancy-induced hypertension. Placental production of erythropoietin is reasonable, because macrophages, rather than cells in the renal interstitium, appear to be the major source in utero,[23] and placental tissue is abundant with macrophages.[24, 25] In support of this speculation, we observed concentrations of erythropoietin in the blood of neonates delivered of women with pregnancy-induced hypertension that were higher than those measured in neonates with Rh-hemolytic disease. However, erythropoietin concentrations were not greater in supernatants of cultured placental cells from hypertensive versus normotensive gestations. Additionally, erythropoietin concentrations were lower than those known to inhibit neutrophil production in vitro.[19] Thus we conclude that erythropoietin is not a likely candidate as the inhibitor of neutrophil production elaborated by placental tissue in pregnancy-induced hypertension.

In summary, the neonatal neutropenia accompanying maternal pregnancy-induced hypertension does not appear to be a function of either reduced placental elaboration of granulocyte and granulocyte-macrophage colony-stimulating factors or a diminished responsiveness of neutrophil progenitors to these factors. However, an inhibitor of neutrophil production is present in cord blood serum and elaborated by the placenta in pregnancy-induced hypertension. We speculate that the identification of this inhibitor may further our understanding of this common maternal disorder and its effects on the fetus.

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Hemorrhage-induced reductions in plasma atrial natriuretic factor in the ovine fetus

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To investigate the effects of blood volume reduction on fetal plasma atrial natriuretic factor concentrations, chronically catheterized ovine fetuses at 109 to 138 days' gestation were subjected either to withdrawal of two consecutive blood samples or to a moderate hemorrhage. In fetuses from which two blood samples of 3.5 ml each (approximately 1% of fetal blood volume) were withdrawn under basal conditions at 15- to 30-minute intervals, plasma atrial natriuretic factor concentrations in the second sample decreased by 17 ± 7 pg/ml from 178 ± 8 pg/ml in the first sample ($p < 0.02$). If the fetal blood removed during the first sample was replaced with an equal volume of maternal blood, plasma atrial natriuretic factor concentrations did not change significantly. In these same samples, plasma arginine vasopressin and angiotensin II concentrations were unchanged between the two samples regardless of whether volume was replaced. In fetuses subjected to hemorrhages of 21% ± 2% over 10 minutes without blood replacement, plasma atrial natriuretic factor concentration at 1.5 hours after hemorrhage was suppressed by 42 ± 10 pg/ml from basal level of 139 ± 9 pg/ml ($p < 0.05$). Plasma atrial natriuretic factor returned to control levels by 5.5 hours after hemorrhage as blood volume returned to normal. Thus in the ovine fetus a reduction in blood volume results in a decrease in plasma atrial natriuretic factor concentrations. Also, atrial natriuretic factor appears to be more sensitive to changes in blood volume than other vasoactive hormones studied. (AM J OBSTET GYNECOL 1991;165:474-81.)

Key words: Blood volume, fetal sheep, atrial natriuretic factor, arginine vasopressin, angiotensin II

Atrial natriuretic factor is present in high concentrations in the fetal circulatory system.[1-3] Intravascular administration of exogenous atrial natriuretic factor significantly affects the fetal cardiovascular system in much the same way it affects the adult cardiovascular system.[4, 5] Recent studies have explored the regulation of atrial natriuretic factor release in the fetus. Of the factors examined thus far, vascular volume expansion was found to stimulate the release of atrial natriuretic factor[6, 7]; this effect was potentiated by fetal hyperosmolality.[8] In addition, hypoxia has been shown to be a potent stimulus for elevating plasma atrial natriuretic factor concentration in the ovine fetus.[9] On the other hand, conditions in which fetal plasma atrial natriuretic factor levels are suppressed have not been described. In the ovine fetus, circulating atrial natriuretic factor concentrations are significantly higher than those in adult sheep,[1, 10] and it is not known whether this high level of atrial natriuretic factor can be reduced under physiologic conditions. Expansion of vascular volume in the fetus increases plasma atrial natriuretic factor concentrations, therefore the reverse situation of blood volume reduction may decrease plasma atrial natriuretic factor levels. To test this hypothesis, we examined the effect of hemorrhage on the normally high plasma atrial natriuretic factor concentration in the ovine fetus.

Material and methods

Pregnant ewes with singleton fetuses were studied in an experimental protocol approved by the University of California, San Diego, committee on animal research. We adhered to the guidelines of the University and the National Institutes of Health for the care and use of these animals. The fetuses were implanted with chronic indwelling arterial, venous, and amniotic fluid catheters. Surgical preparation, care, and postoperative antibiotic regimen have been described in detail elsewhere.[6] The experiments began 5 or 6 days after surgery when the fetal gestational age was 109 to 138 days (term = 145 to 150 days). During the experiment fetal arterial pressure, venous pressure, heart rate, and amniotic fluid pressure were continuously recorded on an on-line computer, as described previously.[11]

To investigate the effect of blood removal because of sampling on plasma atrial natriuretic factor concentra-

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tions, 104 animals were studied. Most animals were studied once, and some were studied twice on two different days, separated by a minimum of 48 hours. Under basal conditions, two consecutive blood samples of 3.5 ml each (an average of approximately 1% of the circulating fetal blood volume) were taken at 15- to 30-minute intervals for atrial natriuretic factor measurements. In one group of fetuses, the blood volume removed for the first sample was not replaced with fluid or maternal blood. In the other group, the volume removed was replaced with an equal volume of maternal blood. In most of these paired blood samples, plasma arginine vasopressin concentrations were also measured. In addition, angiotensin II concentrations were measured in some of these paired samples. Other data obtained from the animals used for the sampling study have been presented in several of our recent reports.[4, 6, 9, 12-16]

In the hemorrhage experiments, 10 fetuses were studied. The protocol consisted of a 30-minute control period, a rapid continuous hemorrhage over 10 minutes, and a 6-hour recovery period. At 24 to 25 hours after hemorrhage, all variables were monitored. During the control period and at 24 hours after hemorrhage, the volume of blood circulating in the fetus was measured with technetium 99–labeled autologous fetal red blood cells.[17] At intermediate times, blood volumes were calculated from the hematocrits after corrections for volume of red blood cells removed in sampling as previously described.[18] Fetal blood samples of 4 ml each were taken at 10 and 30 minutes during the control period, at the end of hemorrhage, and at 1.5, 3.5, 5.5, 24, and 25 hours after hemorrhage for measurements of fetal blood gases and pH (blood gas analyzer model 1302, 39.5° C, Instrumentation Laboratory, Lexington, Mass.), microhematocrit in triplicate, and plasma atrial natriuretic factor concentrations. Additional blood samples of 1 ml each were taken at 20 minutes during the control period and 2.5, 4.5, 6.5, and 24.5 hours for hematocrit determinations. Parts of the results on fetal blood volume restoration, hematocrits, and cardiovascular changes after hemorrhage have been presented in detail elsewhere.[14]

The concentration of atrial natriuretic factor in the plasma was measured by radioimmunoassay methods previously described.[6] The plasma samples were extracted with reverse phase C_{18} microcolumns (Sep-Pak, Waters Associates, Milford, Mass.). The recovery of the extraction procedure determined by the addition of 100 pg atrial natriuretic factor to fetal plasma averaged 91%. The extracted samples were reconstituted in buffer, and atrial natriuretic factor concentrations were measured with an antibody generated against human atrial natriuretic factor 1-28 (Peninsula Laboratories, Belmont, Calif.). Human atrial natriuretic factor 1-28 was used for the standard and the tracer. The interassay and intraassay coefficients of variation were 9.5% and 4.5%, respectively. Plasma arginine vasopressin and angiotensin II concentrations were measured by radioimmunoassay procedures reported previously.[6, 12]

The data are presented as mean ± SE. Each variable was analyzed for significance in changes with time by using a two-way analysis of variance for repeated measures, with time and animal as the two factors. If the changes were significant, the individual means were compared using Fisher's least significant difference test for multiple comparisons. To explore relationships between variables, standard bivariate and multivariate linear regression analyses were performed. The paired Student t test was used to analyze hormone concentrations in the two consecutive blood samples under basal conditions.

Results

Effect of blood sampling. In two groups of fetuses between the gestational ages of 109 and 138 days, two consecutive blood samples of 3.5 ml each were taken under basal conditions at 15- to 30-minute intervals. In the group in which the blood withdrawn for the first sample was not replaced with fluid or maternal blood, plasma atrial natriuretic factor concentration decreased significantly from the first to the second sample by 17 ± 7 pg/ml, a 9.6% increase ($p < 0.02$, Table I). In the group in which the blood removed during the first sample was replaced with an equal volume of maternal blood, plasma atrial natriuretic factor concentration did not differ from the first to the second sample ($p = 0.84$). In both groups of fetuses, neither plasma arginine vasopressin nor angiotensin II concentrations changed significantly from the first to the second sample (Table I). There were no significant differences in the concentration of the first sample between the two groups for each of the three hormones.

Effect of hemorrhage. In the 10 fetuses at 113 to 134 days' gestation, an average of 21.0% ± 1.7% of their initial blood volume (range 13.5% to 29.8%) was removed over a 10-minute period. Control fetal blood volume was 110.8 ± 1.8 ml/kg. At the end of hemorrhage, blood volume was reduced to 93.6 ± 2.3 ml/kg, a 15.0% decrease ($F = 18.6, p < 0.0001$, Table II). Blood volume remained significantly below control levels for 5.5 hours after hemorrhage. At 6.5 hours after hemorrhage, fetal blood volume returned to levels not significantly different from the control levels (Fig. 1).

Mean plasma atrial natriuretic factor concentration in the fetuses during the 30-minute control period before hemorrhage was 139 ± 9 pg/ml (Table II). The atrial natriuretic factor concentration in the second

Table I. Effect of blood sampling on plasma hormone concentrations in ovine fetuses from 109 to 138 days' gestation

	No volume replacement				*Volume replacement*			
	n	*Sample 1*	*Sample 2*	*Δ*	*n*	*Sample 1*	*Sample 2*	*Δ*
ANF	131	178 ± 8	161 ± 7*	−17 ± 7*	36	195 ± 12	197 ± 13	+1 ± 7
AVP	134	7.5 ± 0.7	7.2 ± 0.7	−0.3 ± 0.5	27	8.3 ± 2	7.8 ± 1.6	−0.5 ± 0.8
ANG II	39	13.9 ± 1.8	15.1 ± 1.9	+1.2 ± 1.1	34	11.9 ± 1.5	12.6 ± 1.5	+0.6 ± 0.6

Plasma concentrations are expressed in picograms per milliliter (mean ± SE). Each blood sample was 3.5 ml; sample 2 was taken 15 to 30 minutes after samples 1 (n = number of paired samples). Immediately after sample 1 was taken, 3.5 ml of maternal blood was reinfused into the fetus. *ANF,* Atrial natriuretic factor; *AVP,* arginine vasopressin; *ANG,* angiotensin.

*$p < 0.02$ by paired Student's t test, as compared with sample 1 in the same group for the same hormone.

Table II. Control and hemorrhage-induced changes in cardiovascular variables in ovine fetuses

			Recovery (hr)	
	Control	*Hemorrhage*	*1.5*	*5.5*
Arterial pressure (mm Hg)	42.3 ± 2.1	32.1 ± 1.5*	41.2 ± 2.2	39.6 ± 2.2†
Venous pressure (mm Hg)	3.2 ± 0.6	2.7 ± 0.7‡	2.9 ± 0.5	3.1 ± 0.5
Heart rate (beats/min)	186 ± 7	158 ± 8*	190 ± 6	191 ± 6
Po_2 (mm Hg)	24.5 ± 0.4	21 ± 1*	23 ± 1‡	22 ± 1†
Pco_2 (mm Hg)	49.5 ± 0.5	52.1 ± 1.1†	51.3 ± 0.8‡	54.6 ± 1.0*
pH	7.379 ± 0.012	7.352 ± 0.015§	7.359 ± 0.011†	7.361 ± 0.012†
Osmolality (mOsm/kg)	299.6 ± 1.0	300 ± 1	301 ± 1‡	301 ± 1
Blood volume (ml/kg)	110.8 ± 1.8	93.6 ± 2.3*	95.4 ± 2.3*	105.5 ± 3.5‡
Plasma ANF (pg/ml)	139 ± 9	110 ± 9‡	97 ± 8§	119 ± 6

Values expressed as mean ± SE in 10 fetuses. *ANF,* Atrial natriuretic factor.

*$p < 0.0001$, as compared with control of same variable.

†$p < 0.01$, as compared with control of same variable.

‡$p < 0.05$, as compared with control of same variable.

§$p < 0.001$, as compared with control of same variable.

control sample was lower than that in the first control sample in each of the 10 fetuses, with the mean decrease being 12.0 ± 2.5 pg/ml ($p < 0.001$). In response to hemorrhage, plasma atrial natriuretic factor concentration decreased significantly to 110 ± 9 pg/ml at the end of hemorrhage and continued to decrease further to 97 ± 8 pg/ml at 1.5 hours after hemorrhage (F = 7.78, $p < 0.0001$). This represents a decrease of 30.2% in plasma atrial natriuretic factor concentrations. Thereafter, atrial natriuretic factor slowly returned to basal level; by 5.5 hours after hemorrhage, plasma atrial natriuretic factor concentration was not significantly different from the control levels (Table II, Fig. 2). To determine the relationship between fetal blood volume and plasma atrial natriuretic factor concentration, bivariate regression analysis was performed with data obtained at the end of hemorrhage. The analysis showed that the hemorrhage-induced decrease in plasma atrial natriuretic factor concentration was significantly correlated with the percentage of reduction in blood volume ($r = 0.665$, $p < 0.05$; Fig. 3).

Fetal cardiovascular parameters during control, hemorrhage, and recovery periods are shown in Table II. Arterial pressure fell significantly at the end of hemorrhage, returned to control levels at 1.5 hours after the hemorrhage, and decreased below control levels throughout the remaining recovery period (F = 7.00, $p < 0.0001$). Fetal venous pressure decreased during hemorrhage and decreased further by 0.7 ± 0.3 mm Hg at 30 minutes after hemorrhage (F = 2.09, $p < 0.05$). This decrease was transient; venous pressure returned to basal levels by 1.5 hours after hemorrhage. Fetal heart rate decreased significantly at the end of hemorrhage (F = 2.90, $p < 0.01$), but returned to control levels 1.5 hours after the hemorrhage. Multivariate regression analysis, showed that there was no correla-

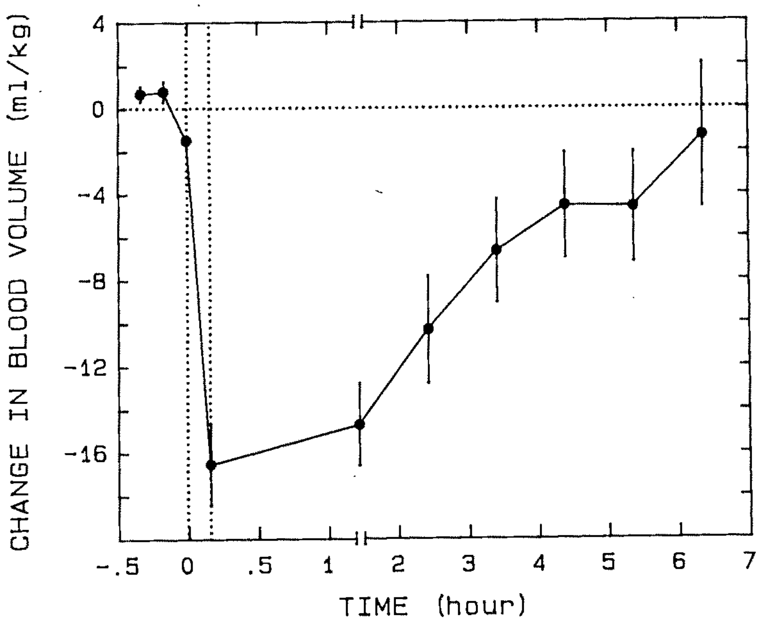

Fig. 1. Hemorrhage-induced changes (mean ± SE, $n = 10$) in blood volume in ovine fetuses at 125 ± 2 days' gestation. Fall in blood volume during and after hemorrhage, analyzed by two-way analysis of variance, was significant (F = 18.6, $p < 0.0001$). *Vertical dotted lines* mark beginning and end of hemorrhage.

tion between the change in plasma atrial natriuretic factor concentration observed at the end of hemorrhage and the change in venous pressure or heart rate.

Fetal blood gas changes during and after hemorrhage are shown in Table II. Arterial Po_2 and pH fell significantly at the end of hemorrhage and remained low throughout the 6-hour recovery period (F = 7.21, $p < 0.0001$ for Po_2; F = 7.64, $p < 0.0001$ for pH). Arterial Pco_2 increased significantly at the end of hemorrhage and was elevated during the entire recovery period (F = 7.64, $p < 0.0001$). Plasma osmolality after hemorrhage underwent a small but significant increase from control levels (F = 2.3, $p < 0.05$). No significant correlation was found between the decrease in plasma atrial natriuretic factor concentration and the change in blood gases, pH, or osmolality (bivariate and multivariate regression analyses).

All 10 fetuses survived the hemorrhage and were alive at the end of the 6-hour recovery period. Three of the fetuses died overnight; at 24 hours after hemorrhage, only seven fetuses were alive. The cardiovascular and blood gas values for these seven fetuses during the prehemorrhage control period and at 24 hours after the hemorrhage are shown in Table III. Arterial pressure, venous pressure, and heart rate at 24 hours after hemorrhage were not different from those during the control period. Plasma osmolality remained within normal range. However, fetal blood gases and pH underwent significant changes at 24 hours. Arterial Po_2 and pH were significantly reduced (F = 11.78, $p < 0.001$; and F = 3.16, $p < 0.05$, respectively) and Pco_2 was elevated (F = 8.23, $p < 0.001$). Surprisingly, fetal blood volume was significantly below control levels (F = 8.23, $p < 0.001$), while plasma atrial natriuretic factor concentration was significantly elevated (F = 5.19, $p < 0.001$) (Table III).

To explore the factors that might have contributed to the increase in plasma atrial natriuretic factor 24 hours after the hemorrhage, multivariate and bivariate regression analyses were performed. The results showed that the increase in atrial natriuretic factor concentration at 24 hours was not significantly correlated with the change in arterial Po_2, osmolality, blood volume, venous pressure, or heart rate.

Comment

The concentration of atrial natriuretic factor in ovine fetal circulation under basal conditions is four to five times higher than that in pregnant adult sheep.[1] Available evidence suggests that the regulation of atrial natriuretic factor release in the fetal animal is essentially similar to that in the adult. Blood volume expansion is a stimulus for elevating plasma atrial natriuretic factor concentrations in the fetus,[6, 7] although the fetal response was more transient than that of the adult.[19] This study demonstrated conversely that blood volume reduction can decrease plasma atrial natriuretic factor concentration, suggesting that blood volume is an important determinant of plasma atrial natriuretic factor concentrations in the fetus.

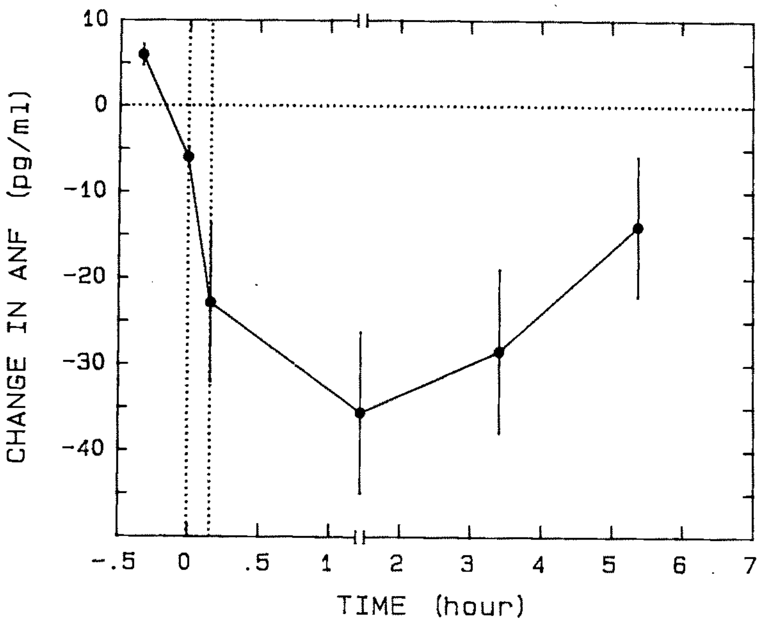

Fig. 2. Change (mean ± SE, $n = 10$) in plasma atrial natriuretic factor concentrations in ovine fetuses during and after hemorrhage. Plasma atrial natriuretic factor at end of and at 1.5 hours after hemorrhage were significantly suppressed from control levels ($F = 7.78$, $p < 0.0001$, analysis of variance). *Vertical dotted lines* mark beginning and end of hemorrhage.

Previous studies have investigated the effects of hemorrhage on plasma atrial natriuretic factor levels in adults of different species. However, the results have been inconclusive in that atrial natriuretic factor levels after hemorrhage were either elevated, suppressed, or unchanged. In a study in human beings,[20] removal of up to 1000 ml of blood over a 30-minute period had no significant effect on plasma atrial natriuretic factor levels; in the sheep, plasma atrial natriuretic factor concentrations did not change during or after a hemorrhage of 15 ml/kg over a 10-minute period.[21]

On the other hand, in pigs in which 40% of the calculated blood volume was removed over a 30-minute period, plasma atrial natriuretic factor concentrations increased significantly even though atrial pressure fell.[22, 23] In contrast, hemorrhage in dogs,[24] rats,[25] and rabbits[26] and blood donation in humans[27] resulted in falling plasma atrial natriuretic factor levels. Consistent with these latter observations, our study in the fetus demonstrated that an acute hemorrhage of 21% of initial blood volume over a 10-minute period significantly reduced plasma atrial natriuretic factor concentrations. At the end of hemorrhage, the decrease in plasma atrial natriuretic factor was significantly correlated with the decrease in fetal blood volume. Blood volume reached the lowest value of 15% of initial blood volume at the end of hemorrhage and remained low for the first 2 hours of the recovery period.

In parallel with this fall in blood volume, plasma atrial natriuretic factor concentrations were lowest during the same time period. With the restoration of fetal blood volume to normal at the end of the 6-hour recovery period, plasma atrial natriuretic factor levels gradually returned to normal. These observations strongly suggest that fetal blood loss was part of the cause for the reduction in plasma atrial natriuretic factor concentrations. However, factors in addition to blood volume appear to be important in that only 42% of the variations in plasma atrial natriuretic factor concentration can be attributed to the blood volume changes as determined by regression analysis (Fig. 3). Findings similar to ours were reported in a recent study in pigs in which a hemorrhage of 14 ml/kg over 5 minutes significantly decreased plasma atrial natriuretic factor levels in the recovery period.[28] Whether the decrease in plasma atrial natriuretic factor concentration was caused by a decrease in secretion or an increase in the clearance rate of atrial natriuretic factor or both was not known, although a reduced secretion seems most likely because of the reduction in venous pressure.

During hemorrhage, fetal arterial pressure decreased and was suppressed during the recovery period. Venous pressure and heart rate fell significantly, but transiently. These changes in vascular pressures were consistent with our earlier report in ovine fetuses subjected to acute hemorrhages of 14% of initial blood volume over a 5-minute period.[18] However blood volume restoration in our study occurred more slowly than in the previous study, possibly because the hemorrhage in our present study was more severe. In addition, blood sampling continued after the hemorrhage at reg-

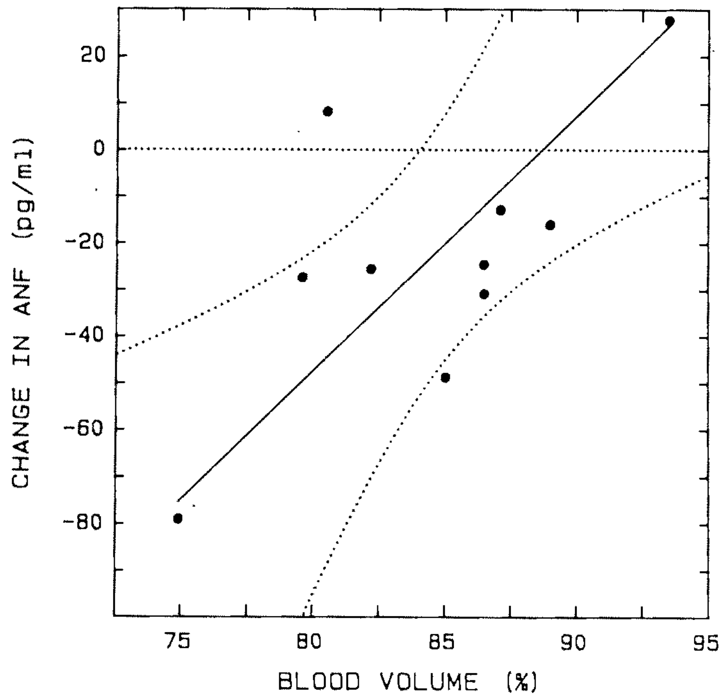

Fig. 3. Correlation between changes in plasma atrial natriuretic factor concentrations and blood volume at end of hemorrhage in ovine fetuses ($r = 0.649$, $p < 0.05$). The 95% confidence interval about the regression line is shown.

ular intervals at a volume of 4 ml per sample, which might cause partial depletion of the volume restored after the initial hemorrhage. A significant relationship between the fall in plasma atrial natriuretic factor levels and the cardiovascular or blood gas changes during hemorrhage was not observed with any of these variables. Furthermore, the gestational age of the fetus had no effect on the changes of these variables during and after hemorrhage. Thus blood volume appeared to be primarily responsible for the decrease in plasma atrial natriuretic factor concentrations during and after hemorrhage. However, the fall in venous pressure during the immediate period after hemorrhage might contribute to the fall in plasma atrial natriuretic factor levels at that time.

Table III. Cardiovascular variables during control and at 24 hours after hemorrhage in ovine fetuses

	Control	*24 hr*
Arterial pressure (mm Hg)	44.3 ± 2.6	42.3 ± 3.2
Venous pressure (mm Hg)	3.7 ± 0.8	3.1 ± 0.5
Heart rate (beats/min)	189 ± 9	188 ± 10
Po_2 (mm Hg)	25 ± 1	20 ± 1*
Pco_2 (mm Hg)	48.8 ± 0.5	56.4 ± 2.6†
pH	7.388 ± 0.017	7.311 ± 0.043†
Osmolality (mOsm/kg)	299 ± 1	300 ± 2
Blood volume (ml/kg)	112.1 ± 1.8	102.9 ± 4.8†
Plasma ANF (pg/ml)	136 ± 11	301 ± 82†

Values expressed as mean ± SE in seven fetuses. *ANF*, Atrial natriuretic factor.

*$p < 0.001$, as compared with control of same variable.

†$p < 0.01$, as compared with control of same variable.

At 6 hours after hemorrhage, all cardiovascular variables, blood volume, and plasma atrial natriuretic factor levels had returned to normal. However, at 24 hours after hemorrhage, fetal blood volume was significantly below control volume, and plasma atrial natriuretic factor concentrations were elevated to 230% of control. Thus plasma atrial natriuretic factor increased even though it had decreased immediately after hemorrhage and even though blood volume was reduced. Under these conditions, factors other than blood volume must be responsible for the elevated atrial natriuretic factor concentrations. A possible explanation for the elevated levels of atrial natriuretic factor at 24 hours is a decrease in renal clearance of atrial natriuretic factor in these hypoxic and acidotic fetuses. Acidemia has been shown to decrease blood flow to the kidney,[29] decreasing renal clearance; however, this would only account for a small portion of the rise in plasma atrial natriuretic factor concentrations because the fetal kidneys contribute minimally to the plasma clearance of atrial natriuretic factor.[30]

In evaluating other factors which might have contributed to the increase in plasma atrial natriuretic factor levels at 24 hours, we have previously reported that hypoxia is a potent stimulus for the release of atrial natriuretic factor in the ovine fetus.[9] In addition, hyperosmolality can directly stimulate the fetal atria to release atrial natriuretic factor and potentiate the effect of vascular volume expansion on atrial natriuretic factor release.[8] However, when these possibilities were explored in the present study using regression analysis, the increase in atrial natriuretic factor at 24 hours was not significantly correlated with the decrease in arterial Po_2 or plasma osmolality. We have also observed an increase in plasma arginine vasopressin concentrations in these fetuses 24 hours after hemorrhage.[14] This rise could stimulate the release of atrial natriuretic factor; however, this possibility is unlikely because pharmacologic concentrations of arginine vasopressin are required for the release of atrial natriuretic factor[31]; the rise in arginine vasopressin observed in these studies was within the physiologic range. Furthermore, arginine vasopressin primarily releases atrial natriuretic factor through its pressor effects; in our studies the vascular pressures were not elevated at any time after hemorrhage.[14]

These findings suggest that, under the condition of blood loss and volume restoration, the regulation of atrial natriuretic factor release could be multifactorial. Whereas volume reduction and decreases in venous pressure tended to lower atrial natriuretic factor release, factors such as reduced renal clearance, hypoxia, and hyperosmolality tended to enhance atrial natriuretic factor release. The observed changes in atrial natriuretic factor levels could be the result of these interactive mechanisms.

To determine the sensitivity of the effect of blood volume on plasma atrial natriuretic factor concentrations, we examined 167 pairs of consecutive fetal blood samples for the effect of blood sampling on plasma atrial natriuretic factor levels under basal conditions. Removal of 3.5 ml of fetal blood without volume replacement (that is, removal of approximately 1% of fetal blood volume) resulted in the significantly decreased plasma atrial natriuretic factor concentrations seen when the blood was resampled 15 to 30 minutes later. However, if the blood was replaced with an equal volume of maternal blood, plasma atrial natriuretic factor concentration remained unchanged during the same time period. In contrast, plasma arginine vasopressin and angiotensin II were not affected by the two sampling procedures regardless of whether blood was replaced. In a previous study in which ovine fetuses were subjected to slow hemorrhage over 2 hours, we found that plasma renin activity, arginine vasopressin, and norepinephrine concentrations increased significantly only after removal of 5% to 10%, 10% to 15%, and 20% to 30%, respectively, of the initial fetal blood volume.[32] In the current study, plasma atrial natriuretic factor concentrations decreased significantly after removal of only 1% of fetal blood volume; this decrease suggests that fetal atrial natriuretic factor may be more sensitive to changes in blood volume than other vasoactive hormones. We are surprised by this finding and have no explanation for the observation that plasma atrial natriuretic factor, but not arginine vasopressin or angiotensin II, was responsive to such small blood volume changes.

In adults, the mechanism responsible for the release of atrial natriuretic factor during blood volume expansion appears to be atrial stretching. The increase in vascular volume elevates venous return, resulting in a rise in right atrial pressure and an increase in atrial stretch, which in turn initiates the release of atrial natriuretic factor from atrial myocytes.[33] Presumably, the same mechanism would operate in the fetus. The finding that a decrease in fetal blood volume lowers plasma atrial natriuretic factor concentrations suggests that atrial natriuretic factor is normally released in the fetus as a result of a stimulated state, which is consistent with the observation that basal plasma atrial natriuretic factor levels in rat,[3] sheep,[1] and human[34] fetuses are significantly higher than such levels in adults of the same species.

Our study suggests that the basal level of atrial natriuretic factor in fetal circulation is normally released as a result of a stimulated state and that fetal plasma atrial natriuretic factor can be reduced by blood volume removal.

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LETTERS TO THE EDITORS

Prediction of survival of infants weighing <800 grams at birth: Errors in the application of multivariate statistical techniques

To the Editors: The development and validation of a practical and accurate prognostic model in extremely low birth weight infants represents an important challenge for modern medicine. In the article by Lipper et al. (Lipper EG, Ross GS, Auld PAM, Glassman MB. Survival and outcome of infants weighing <800 grams at birth. AM J OBSTET GYNECOL 1990;163:146-50) the authors have presented a multivariate predictor of mortality in extremely low birth weight infants. Unfortunately, there are several important methodologic flaws in their model. Fully 21 "independent" variables were entered into the multiple logistic regression equation examining 80 inborn extremely low birth weight infants, with an unspecified number of outcome events (i.e., deaths). No univariate analysis comparing differences in these 21 variables between survivors and nonsurvivors was presented. To support a multivariate examination of 21 variables, between 105[1,2] and 210[3] outcome events (deaths) would have been required. We are told that 41 of all 184 extremely low birth weight infants admitted to the Neonatal Intensive Care Unit were discharged home, so we know that 77.7% died. Therefore we can estimate that approximately 62 of the 80 inborn infants died (i.e., there were approximately 62 outcome events).

Clearly, the sample size is too small to support the type of analysis reported. Furthermore, the authors state that the "independent variables were examined for multicollinearity and no serious collinearity problems were found." It is difficult to accept this statement at face value because there is a high degree of correlation between many of the infant variables included (e.g., length of gestation, birth weight, crown-heel length, and head circumference). For example, we have previously reported a correlation as high as 0.87 (Pearson correlation coefficient) between birth weight and crown-heel length in infants who weigh <1500 gm at birth[4] and we would not be surprised if there were a similar high correlation between 5-minute Apgar score and initial pH value.

In their discussion of other prognostic models, the authors erroneously comment that "the definition of viability was not specified" in the article by Ales et al.[4] The methods section of that article[4] plainly states that all live-born singleton infants who weighed ≤1500 gm at birth were included with the exception of six infants "with abnormalities incompatible with life (i.e., Potter's syndrome, anencephaly, encephalomyelomeningocoele, thanatophoric dysplasia syndrome, huge pelvic and sacrococcygeal teratomas)."[4] Table II in that article[4] documents that 22% (36/158) of the infants in the derivation data set weighed ≤800 gm at birth. Infants who died soon after delivery were not excluded. Because this was not a prospective study and uniformity of timing or circumstances for obtaining initial pH value could not be assured, initial pH value was not considered in the multivariate model. However, the model, based on birth weight and 5-minute Apgar score, had a predictive accuracy of 84.5% and was validated in a separate cohort of very low birth weight infants.

Although initial pH value and 5-minute Apgar score intuitively sound like reasonable candidates for significant predictors, the stability of the multivariate model reported in Lipper's article is of enormous concern, given the number of variables considered and the high correlations between many of the infant variables. We suggest that the authors reexamine their data after studying the univariate differences between survivors and nonsurvivors and clarifying correlations among all variables being considered for the multivariate model. Only then should they select at most six to 12 truly independent variables for inclusion in their multivariate model. Finally, it is of the utmost importance to validate prognostic models in separate data sets.

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Reply

To the Editors: Our study represented the cumulation of 5 years of data (1980 to 1985) in which we were able to examine the effect of maternal and perinatal variables on mortality in 80 inborn extremely low birth weight infants. Ideally, we would have preferred a larger sample for what we intended to be the exploratory phase of our study. We are currently compiling data for the second half of the 1980s, which should enable us to validate the predictor model in a second, confirmatory (i.e., independent) data set.

In terms of the examination of our variables for multicollinearity, we stand true to our statement that no serious collinearity problems were found. The restriction of the population under investigation to a birth weight of 500 to 799 gm results in a reduction in the variance of other independent variables. Therefore the correlations among these variables were not suggestive of collinearity, with the exception of the Apgar score at 1 and 5 minutes in which the correlation was 0.78, which would of course be expected. The next largest correlation was between head circumference and gestational age (0.65); no other correlation was higher than 0.56.

Finally, we did not intend to misquote the article by Ales et al. It was not clear from the manuscript whether infants <800 gm who died shortly after birth were excluded. Now this point has certainly been clarified by the authors.

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A plea for early diagnosis of fallopian tube carcinoma: Can hysteroscopy be of use?

To the Editors: Primary carcinoma of the fallopian tube is the rarest genital malignancy, constituting 0.2% to 1% of all gynecologic cancers.[1] Hence, definitive preoperative diagnosis is rarely made and is often confused with ovarian cancer. More importantly, the diagnosis is delayed until the disease is advanced. Prognosis depends on the stage of disease.[1, 2] Early definitive diagnosis of fallopian tube carcinoma has received relatively scant attention. Tubal carcinoma, unlike ovarian, arises in a hollow viscus with muscular walls and becomes symptomatic earlier, with vaginal bleeding or discharge in 50% and pain in 30%.[1] Only 14% of patients did not have symptoms in a recent series.[2]

For early diagnosis, vaginal pool cytology is of limited value.[1] Hirai et al.[3] reported positive endometrial aspiration cytology in 60% of patients and have made a plea for repeated sampling. Also, laparoscopy has been suggested in women with postmenopausal bleeding and negative diagnostic curettage.[1] Vaginal ultrasonography with its improved resolution can be expected to detect many more tubal lesions, which should be investigated by laparoscopy.

Another commonly performed investigation in postjenopausal and dysfunctional bleeding is hysteroscopy. With the disease being extremely rare, no individual gynecologist can be expected to collect sufficient information in this regard, hence there is need for a uniform protocol. Will it be possible to suspect or diagnose tubal carcinoma on hysteroscopy? It may identify the source of abnormal discharge from the tubal openings. Salpingoscopy is limited to the exploration of the initial 5 mm of the tube (tubaloscopy) but it should certainly be possible to take aspiration from the intramural portion of tubes or tubal openings for cytology. This, in addition to endometrial aspiration and posterior vaginal pool samples, would increase the detection rate of malignant cells from tubal carcinoma. Therefore, it is suggested that particular attention be paid to tubal openings and aspiration samples be taken from these openings during hysteroscopic evaluation of abnormal bleeding when uterine cavity shows no lesion. Can we expect early definitive diagnosis of fallopian tube carcinoma in this era of endoscopy?

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Egg transport through the oviduct

To the Editors: In his letter on the nature of egg transport through the oviduct Dr. Ch. Egarter (Egarter C. The complex nature of egg transport through the oviduct. AM J OBSTET GYNECOL 1990;163:687), referring to an article in the JOURNAL (Pulkkinen MO, Jaakkola U-M. Low serum progesterone levels and tubal dysfunction—A possible cause of ectopic pregnancy. AM J OBSTET GYNECOL 1989;161:934), hypothesizes that an egg can enter the contralateral fallopian tube via the uterus. In their reply, Drs. Pulkkinen and Jaakkola assert that this mechanism seems unlikely and is not supported by experimental findings.

This may be true; however, the possibility of this uncommon mechanism is supported by clinical observations. According to Marchesi[1] the ovum may sometimes pass through the fundal region of the uterus and enter the contralateral oviduct (the author calls this phenomenon "internal migration"). That is the only possibility to explain the observed cases of ectopic pregnancies implanted in an oviduct whose distal extremity was certainly occluded or even had been resected during a previous surgery. Although this is an uncommon event, it cannot be excluded.

This confirms that the nature of the transport of the egg through the oviduct is very complex and many factors that are not well known today are involved.

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Response declined

Relaxation of the human uterine artery by atrial natriuretic peptide is independent of the endothelium

To the Editors: Atrial natriuretic peptide is a 28 amino acid peptide produced in the right atrium and known to induce diuresis, vasorelaxation, and hypotension.[1] An intact vascular endothelium is necessary for the vasodilation induced by acetylcholine, and this relaxation of vascular smooth muscle is mediated by the release

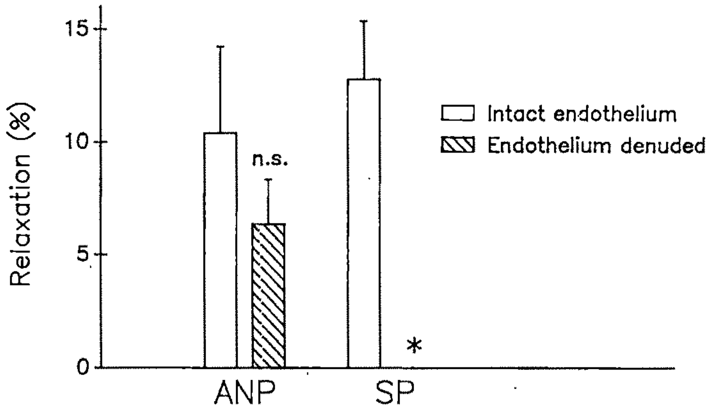

Fig. 1. Induction of relaxation by atrial natriuretic peptide and substance P in vessels with endothelium intact and denuded.

of the so-called endothelium-derived relaxing factor,[2] now identified as nitric oxide. Other signal substances that act by means of endothelium-derived relaxing factor to induce vasodilation are adenosine triphosphate, substance P, bradykinin, and histamine. On the other hand, calcitonin gene-related peptide and vasoactive intestinal polypeptide have been shown to relax vascular smooth muscle independently of an intact endothelium. This investigation was undertaken to elucidate whether atrial natriuretic peptide–induced relaxation of the human uterine artery is related to the vascular endothelium.

Preparations were obtained from eight women (mean age 47 years, range 34 to 64 years) undergoing abdominal hysterectomy for benign gynecologic diseases. The patients were informed of the investigation and had given their consent to excision of the preparations. The uterine arteries were cut into circular preparations that were mounted in organ chambers, containing Krebs-Ringer solution. The mechanical activity was recorded by means of a Grass FT03C force-displacement transducer on a Grass model 7D polygraph (Grass Instruments Co., Quincy, Mass.). The preparations were contracted by exchanging the sodium chloride in the bathing fluid with potassium chloride, and when a stable contraction was established atrial natriuretic peptide (10^{-7} mol/L) was added to the organ chamber. After registration of possible effects the preparations were washed. Then the endothelium was removed from some of the vascular preparations by twice gently injecting the oxygen-carbon dioxide gas mixture through the vessel lumen with a 0.3 mm cannula during 5 to 10 minutes at an interval of 10 minutes. The correlation between the removal of endothelium and the failure of the artery, precontracted by potassium, to relax when exposed to substance P (10^{-7} mol/L) was very strong. Hence absence of the relaxatory effect of substance P was taken as proof of successful removal of the endothelium. After the vessels had been denuded of their endothelium the effect of atrial natriuretic peptide (10^{-7} mol/L) was tested again. The Wilcoxon signed rank test was used to evaluate possible significant differences.

Atrial natriuretic peptide reduced smooth muscular tone in vessels precontracted by potassium chloride ($p < 0.05$). The relaxation was 10.4 ± 3.85% of the potassium-induced contraction ($n = 8$). After removal of the endothelium the isolated vessels still responded with a contraction to potassium chloride depolarization, indicating no damage to the smooth musculature. Both preparations with an intact endothelium and those denuded of it responded with relaxation when exposed to atrial natriuretic peptide. There was no significant difference between the effect of atrial natriuretic peptide in those two types of isolated uterine arteries. In contrast, substance P failed to induce relaxation in vessels from which the endothelium had been removed. The results are illustrated in Fig. 1.

In conclusion, atrial natriuretic peptide relaxes the vascular smooth musculature of the isolated human uterine artery by an endothelium-independent mechanism, which does not involve endothelium-derived relaxing factor, recently characterized as nitric oxide.

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Breast disease management in an obstetrics and gynecology department

To the Editors: We read with great interest the article by Hall et al. (Hall JA, Hall BR, Murphy DC, Hall KA.

Surgical management of breast disease in an obstetrics and gynecology group. AM J OBSTET GYNECOL 1990;162:1526-30), and we would like to express our opinion on this subject and to describe our Breast Unit, which is part of the 1st Obstetrics and Gynecological Department, University of Athens, and is staffed by gynecologists.

We agree that breast diseases can be diagnosed by each doctor who first examines a woman. Breast examination is routine for gynecologists, who are more familiar than other doctors with the philosophy of screening tests, such as Papanicolaou tests. They also have adequate surgical skills. Therefore in our opinion gynecologists are able to offer both diagnostic and surgical treatment of breast diseases. However, we think that breast cancer treatment must be done by a cancer specialist, who deals exclusively with oncology and can be gynecologist-oncologist or surgical oncologist. This person is responsible for the patient's follow-up and for collaborating with the radiotherapist or chemotherapist as a team. Regarding the type of primary operation, breast conservation procedures can be effectively accomplished only in specialized units, because they involve accurate evaluation of a variety of clinical and biologic factors affecting the mode of cancer treatment.

Our unit was founded in 1979. There is an outpatient department for routine breast examination and follow-up of patients. Mammography is provided by the hospital's radiology group and mammograms are reviewed by our group.[1] Radiotherapy is performed by the hospital radiotherapists, but chemotherapy, when required, is performed by us. During these years 2490 breast biopsies were done and 830 cases of breast cancer were identified and operations were performed. Our treatment of choice for breast cancer is a conservation procedure, when appropriate, for better cosmetic results. Of course, we perform modified radical or radical mastectomy in other cases. In all cases a complete axillary node dissection is performed at three levels. The total number of dissected nodes is 32.59 ± 9.43 (mean ± SD) and their distribution is 22.86 ± 7.09 at low level, 3.28 ± 3.51 at mid level, and 6.25 ± 4.26 at apical level.

In conclusion, a gynecologist, when appropriately trained, can offer primary care for the breast, as well as surgical treatment and follow-up of breast cancer.

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REFERENCE

1. Breast disease programs in obstetrics and gynecology: a plea for training in mammography. AM J OBSTET GYNECOL 1989;161:267-70.

Reply

To the Editors: I would agree with Keramopoulos et al. that the gynecologist can offer primary care and surgical treatment for breast disease. With the new American Cancer Society data stating that as many as 1 in 9 American women may have breast cancer in their lifetime, it is obvious that the gynecologist needs to become involved.

I would disagree that the surgeon performing breast cancer treatment needs to limit his practice to this field. I believe that the surgery should be performed by an adequately trained surgeon, and I feel that is within the expertise of the gynecologic surgeon. Obviously training programs need to be established to accomplish this goal.

Axillary lymph node dissection is performed for staging purposes only. The identification of malignant lymph nodes in the axilla indicates systemic disease, and removal of those lymph nodes is not necessarily a curative operation. Therefore the number of lymph nodes removed in an adequate sample needs to be only enough to provide information on staging. Five to 10 axillary nodes carefully examined by the pathologist will provide as much information as removal of 25 to 30 lymph nodes. The patient with malignant axillary nodes has no better chance of survival if she has a large number of lymph nodes removed than if she has only a few lymph nodes removed. The importance of the axillary node dissection is only to identify the existence of spread of the cancer into the lymphatics. An axillary node dissection that does not yield malignant nodes fails to guarantee the absence of metastatic disease. Axillary node dissection with large numbers of lymph nodes removed, as mentioned by Keramopoulos et al. (approximately 32), indicates thoroughness but removes more axillary tissue than is necessary for staging and greatly increases the complexity of the operation. Removal of a large number of lymph nodes also may increase complications from arm edema and may restrict the radiotherapist from treating the axilla. A dissection of level I nodes can be performed readily and yields an adequate staging sample of approximately 10 nodes without a great risk of long-term adverse sequelae to the patient.

It is time for the gynecologist to become more involved in breast disease. It is also time to recognize that early diagnosis is necessary for survival because of the systemic nature of breast cancer very early in its development.

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Untreated luteal phase deficiency as another possible risk factor for preterm delivery

To the Editors: We read with interest the article by McGregor et al. (McGregor JA, French JI, Richter R, et al. Antenatal microbiologic and maternal risk factors associated with prematurity. AM J OBSTET GYNECOL 1990;163:1465-73). Risk factors for prematurity included infectious causes, maternal medical problems, antepartum treatment for urinary tract infection, prior preterm delivery, bacterial vaginosis (all of which were positively associated with preterm delivery), and factors of maternal age, prior abortion (therapeutic or spontaneous), and fetal sex. Although only 9 (4.5%) of 202 women were delivered at <37 weeks, certain small groups seemed to be associated with higher risks: women aged 30 to 38 years (2/20, 10%), prior preterm birth (4/21, 19%), and *Mycoplasma hominis* infection (3/18, 17%).

We evaluated 540 women who had preterm histories of luteal phase defects but who were eventually able to conceive; 82 (15%) of 540 were delivered at <37 weeks' gestation. This relatively high rate (compared with 4.5% in the study of McGregor et al.) might be attributed to a relatively older group of women (mean age 32 years). We divided the 540 patients into three groups: 32 patients who refused the suggested progesterone therapy (group 1), 469 women who had progesterone therapy for ≥12 weeks (group 2), and 39 patients who took progesterone for ≥6 but <10 weeks (group 3). The rates of prematurity (<37 weeks) were 31.2% for group 1, 13.7% for group 2 ($p < 0.05$ comparing groups 1 and 2), and 10.3% for group 3. The rates of severe prematurity (<32 weeks) were 12.5% for group 1, 1.3% for group 2 ($p < 0.01$ comparing groups 1 and 2), and 2.6% for group 3. The number (mean ± SE) of days delivered early was −16.0 ± 26.8 for group 1, compared with −7.3 ± 13.0 in group 2 and −5.5 ± 16.1 in group 3 ($p < 0.01$ comparing group 1 with either group 2 or group 3). No significant differences (mean ± SE) were found in birth weights (3113 ± 874 gm for group 1, compared with 3240 ± 679 gm for group 2 and 3440 ± 646 gm for group 3).

These data, we believe, support the addition of luteal phase defects to the list of risk factors for preterm delivery. Depending on the population studied, progesterone therapy during the first trimester could prove to be one method of preventing preterm delivery.

Jerome H. Check, MD, and Grace Lee, MD
Division of Reproductive Endocrinology/Infertility, Department of Obstetrics and Gynecology, University of Medicine and Dentistry of New Jersey/Robert Wood Johnson Medical School at Camden, Cooper Hospital/University Medical Center, Three Cooper Plaza, Second Floor, Suite 211, Camden, NJ 08103

Reply

To the Editors: We appreciate the interest of Check and Lee in our prospective study of microbial factors associated with preterm (<37 weeks' gestation) birth. As noted in the article, the low rate of preterm birth (4.5%) is likely explained by our exclusion criteria: patients with recognized risks of preterm birth were excluded in this prospective cohort study of young, otherwise healthy women. Our results confirm the hypothesis that reproductive tract infection and subsequent inflammation are associated with preterm labor and rupture of membranes in some women. The results of a series of controlled trials have recently been presented.[1, 2] We calculate an overall population-attributable risk of approximately one third for preterm birth caused by infection among our patients. Further studies are required to determine which women at risk for preterm birth can benefit from indicated antimicrobial treatment or prophylaxis.

We assume that there are many other causes of preterm birth and that these should be appropriately investigated. Because of progesterone's primary endocrine role in pregnancy, interest in progesterone or progestin treatment during pregnancy is long-standing. Nevertheless, a recent metaanalysis of 20 controlled trials failed to demonstrate benefits.[3] These authors recommended that further controlled trials be limited to pregnant women with demonstrable endocrinologic abnormalities. The study of selected women (those with prior putative luteal phase defects) reported by Check and Lee demonstrated a 15% preterm birth rate, which is similar to that in unselected women at many of the publicly supported clinics we serve. We hope Check and Lee present their complete results soon.

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and Janice I. French, CNM, MS
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Rapid enzyme-linked immunoassay tests for group B streptococcus infection

To the Editors: The article by Towers et al. (Towers CV, Garite TJ, Friedman WW, Pircon RA, Nageotte MP. Comparison of a rapid enzyme-linked immunosorbent assay test and the Gram stain for detection of group B streptococcus in high-risk antepartum patients. AM J OBSTET GYNECOL 1990;163:965-7) again illustrates that we have no adequate means of routine, meaningful antepartum surveillance for group B streptococcus. The shortcomings of the rapid enzyme immunoassay test are substantiated by work done at this university. In a group of approximately 100 patients, the rapid enzyme immunoassay test was used in a double-blind manner very similar to that of Towers et al. Our results were even less optimistic than theirs: When compared with the standard culture for group B streptococcus, the test was only 21% sensitive and showed a 66% specificity, with a positive predictive value of 60%. In our opinion this test has little value in identifying antepartum patients who carry the group B streptococcus bacteria.

David E. Green, MD, G. Wright Bates, MSIV, and Stephen P. Emery, MSIV

Department of Obstetrics and Gynecology, University of Alabama School of Medicine, Huntsville Program, 109 Governors Dr. SW, Huntsville, AL 35801

Response declined

Relationship between central venous pressure and pulmonary capillary wedge pressure in severely toxemic patients

To the Editors: In treating patients with severe toxemia Fliegner[1] emphasized fluid volume expansion to raise central venous pressure to a range between 5 and 10 cm H_2O. However, Clark[2] has observed an inconsistent relationship between central venous pressure and left-side heart pressures as estimated by pulmonary capillary wedge pressure, suggesting that it is dangerous and simplistic to rely on central venous pressure alone for fluid management in patients with severe preeclampsia.

In our intensive care unit we took 623 central venous pressure and pulmonary capillary wedge pressure measurements with a pulmonary artery catheter in 60 patients with severe preeclampsia. Of these measurements 123 were taken without treatment or fluid management (basal), and 500 were taken after hypotensor treatment (clonidine hydrochloride or diazoxide). The correlation values are shown in Table I.

Our data show acceptable values for both correlation and linear regression between central venous pressure and pulmonary capillary wedge pressure under both conditions. However, the variability of correlation values within a single patient is enormous: for a central venous pressure of 4 mm Hg, the corresponding pulmonary capillary wedge pressure values were between − 1.7 and 17.5 mm Hg. This tends to support Clark's assessment that the exclusive use of central venous pressure for fluid management in these patients is inadequate and potentially dangerous, especially if there appear to be oncotic pressure alterations with resulting pulmonary edema.[3] Our results suggest that the correlation between central venous pressure and pulmonary capillary wedge pressure is valid only when considered in a group of patients because of the variability of individual values.

Ramon Tellez, MD, and Roberto Curiel, MD

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Table I. Correlation between central venous pressure and pulmonary capillary wedge pressure

Conditions	*n*	*CVP (mm Hg)*	*PCWP (mm Hg)*	*r*	*p*
Basal	123	4.0 ± 3.0	7.9 ± 4.5	0.651	0.001
Posttreatment	500	4.1 ± 3.4	7.9 ± 4.6	0.647	0.001
TOTAL	623	4.1 ± 3.3	7.9 ± 4.6	0.646	0.001

CVP, Central venous pressure; *PCWP,* pulmonary capillary wedge pressure.

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Prolonged exposure to wood preservatives induces endocrine and immunologic disorders in women

To the Editors: The toxic effects of polychlorinated organic compounds are associated with spontaneous abortions, fetal malformations, growth retardation, stillbirths, thymic degeneration, reduced fertility, and changes in the menstrual cycle.[1-9]

During the postwar economic boom, wood coated with polychlorinated organic compounds containing preservatives was used for ceilings and wall panels of homes. Carpets and leather upholstery impregnated with moth repellants contained the same organic compounds. Pentachlorophenol and the γ-isomer of hexachlorocyclohexane (lindane) were thus brought into homes during modernization. The steady emission of these vapors into today's highly insulated, draft-proofed homes raises the specter of prolonged exposure because these toxins can be incorporated by inhalation, oral uptake, and skin contact. Pentachlorophenol and lindane are easily measured by capillary chromatography and mass spectroscopy. We determined whether these toxins are present in the blood of women referred for endocrinologic evaluation.

We examined 90 women with histories of habitual abortion ($n = 30$), unexplained infertility ($n = 20$), menstrual disorders ($n = 20$), or climacteric symptoms ($n = 20$). In 22 women elevated pentachlorophenol (>25 μg/L) or lindane (>100 ng/L) levels, or both, were found. This group included 8 infertile women, 6 with histories of repeated abortions; 8 women with menstrual disorders; and 6 women with climacteric symptoms and menstrual irregularities complicated by depression. The duration of exposure was shortest in infertility (mean = 4.6 years), followed by menstrual disorders (mean = 6 years) and climacteric symptoms (mean = 10 years). Pentachlorophenol and lindane were highest in women with infertility (pentachlorophenol mean = 73 μg/L, lindane mean = 250 ng/L), lower in those with menstrual dysfunction (pentachlorophenol mean = 42 μg/L, lindane mean = 210 ng/L), and lowest in patients with climacteric symptoms (pentachlorophenol mean = 48 µg/L, lindane mean = 120 ng/L). The source of the toxic vapors was identified in the residence of each patient. The pentachlorophenol concentrations in wood varied from 50 to 4000 mg/kg, and lindane concentrations were between 10 and 600 mg/kg.

Four days after the patients' spontaneous or induced menstruation we determined the concentration of follicle-stimulating hormone, luteinizing hormone, estradiol-17β, and prolactin. Thyroid (protirelin), adrenocortical (corticotropin, purified), and lymphocyte function were evaluated.

Seventeen (77%) of the 22 women were initially seen with adrenocortical malfunction: insufficiency was associated with subnormal dehydroepiandrosterone sulfate (<800 ng/ml) levels in all patients. Six (27%) women were initially seen with thyroid gland dysfunction. Immunologic disorders were observed in 15 (71%) of 21 women. In the 68 women with normal pentachlorophenol and lindane concentrations 35% (32%) were initially seen with adrenocortical malfunction (reduced lymphocyte function).

The source of intoxication was eliminated from the environments of 12 women; the exposure persisted for the remaining 10. All women received hormonal treatment and immunologic stimulation. Symptoms subsided in the 12 women with reduced polychlorinated organic compounds exposure. Menstruation normalized in all 12 patients, three of whom gave birth to healthy babies. In the 10 patients with continued exposure, blood polychlorinated organic compounds concentrations were unchanged during 2 years of observation, and treatment improved symptoms in only two. One patient conceived and had her fifth missed abortion.

We suggest that endocrine and immunologic disorders in women should alert physicians to possible exposure to pentachlorophenol and lindane. The accuracy of this diagnosis should become apparent when the familial and occupational history is reviewed. Prolonged exposure to these environmental toxins may be a factor in hirsutism, alopecia, unexplained infertility, and repeated abortions.

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Pregnancy and Wilson's disease

To the Editors: The report by Dupont et al. of their case of Wilson's disease complicating pregnancy (Dupont P, Irion O, Beguin F. Pregnancy in a patient with treated Wilson's disease: A case report. AM J OBSTET GYNECOL 1990;163:1527-8) was most interesting. I recently managed two similar cases. One of the patients received treatment with zinc sulfate. This case has been reported previously.[1] The baby was delivered at 37 weeks and weighed 2700 gm at birth (25th percentile). The other was a 24-year-old primiparous patient who was known to have Wilson's disease; she was maintained on a regimen of 250 mg penicillamine four times daily. She was without symptoms at the time of conception and remained so throughout the course of pregnancy, labor, and puerperium. However, the fetus was noticed to be small and required close antenatal monitoring by nonstress tests and ultrasonographic examinations. She underwent an emergency cesarean section at 37 weeks' gestation because of fetal distress and a male baby weighing 1560 gm was delivered.

There are few reports of successful pregnancies in women with untreated, symptomatic Wilson's disease. However, from these case reports, we may conclude that those patients who received treatment and are without symptoms may be able to conceive, and a successful outcome of pregnancy is possible. In contrast to the patient described by Dupont et al., both of my patients had small babies. The fetuses of mothers with Wilson's disease should be regarded as high risk and should be closely monitored.

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Reply

To the Editors: We thank Dr. Chin for his interest in our case report and we wish to comment on his remark about small babies of mothers with Wilson's disease.

We think that an expression such as "small baby" used to qualify an infant born at 37 weeks' gestation with a birth weight of 2700 gm (25th percentile) is misleading. Terms such as low-birth-weight infant or small-for-gestational-age infant should be reserved for infants born with a birth weight below 2500 gm or below the 10th percentile according to the sex, thus permitting identification of newborns at risk for neonatal complications.

Dr. Chin's second case (a male newborn weighing 1560 gm at 37 weeks' gestation) could be the first report of intrauterine growth retardation (IUGR) in a pregnancy of a patient with penicillamine-treated Wilson's disease. To date >120 pregnancies in mothers with Wilson's disease have been related. Thirty-one of these pregnancies were in untreated patients[1-4] and the remaining pregnancies were in patients treated with various chelating agents: BAL, zinc sulfate, triethylenetetramine, and especially penicillamine. No mention of IUGR is made in these reports, and all birth weights published are ≥2500 gm, except in one patient (case K.K., a normal infant weighing 2140 gm at term) reported in Walshe's series[5] of patients with triethylenetetramine-treated Wilson's disease. There is one report[6] of IUGR in a patient receiving penicillamine. She had rheumatoid arthritis and was on a regimen of 900 mg penicillamine per day. She was delivered of an abnormal male infant (lax skin, flat face, low ears, flexion contracture, inguinal hernia) weighing 2000 gm at 37 weeks' gestation; he eventually died of septicemia after abdominal surgery. In his series Walshe[5] compares the birth weights of infants of "wilsonian" mothers treated with triethylenetetramine or penicillamine. The results show a marginally higher birth weight in the triethylenetetramine group (9 patients) than in the penicillamine group (35 patients) (2900 vs 2800 gm).

Even though data in the literature are incomplete, it seems that the risk of IUGR in pregnancies of patients with treated or untreated Wilson's disease (two cases known in 120 pregnancies) is below the 3.0% to 10% risk of IUGR generally expected in the normal obstetric population. Nevertheless, in the absence of clinical studies proving the harmlessness of chelating agents in fetal development, we agree with Dr. Chin's final statement, that pregnancies in women with Wilson's disease and their fetuses should be considered as high risk and thus both should be very closely monitored.

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Breast Cancer—Reducing Your Risk if It Runs in Your Family. Edited by Mary Dan Eades, MD. 144 pages. New York, 1991, Bantam Books. $8.95.

Breathing Easy: A Parent's Guide to Dealing With Your Child's Asthma. Edited by Maryann Stevens and Preface by R. Michael Sly. 215 pages. New York, 1991, Prentice Hall Press. $9.95 (paperback).

The Climacteric Hot Flush: Progress in Basic and Clinical Pharmacology. Edited by P. Lomax and E.S. Vesell. 166 pages, illustrated. Basel, Switzerland, 1991, S. Karger AG. $127.25 (U.S.).

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Drug Information for the Health Care Professional. Volumes IA and IB. Eleventh edition. United States Pharmacopeial Convention, Inc. 162 pages. Kingsport, Tennessee, 1991, Arcata Graphics. $110.

Dysfunctional uterine bleeding. Volume II. Edited by R.W. Shaw. 164 pages. Park Ridge, New Jersey, 1990, The Parthenon Publishing Group Inc. $68.

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Heart Disease—Reducing Your Risk if It Runs in Your Family. Edited by Charles Klieman and Kevin Osborn. 162 pages. New York, 1991, Bantam Books. $8.95.

The Management of Infertility. Edited by John Yovich and Gedis Grudzinakas. 276 pages, illustrated. Oxford, 1990, Heinemann Medical Books. $135.

Nursing Care of the Childbearing Family. Edited by Laurie N. Sherwen, Mary Ann Scoloveno, and Carol Toussie Weingarten. 1322 pages, illustrated. Norwalk, Connecticut, 1991, Appleton & Lange. $52.95.

Overcoming Infertility. Edited by Robert Nachtigall and Elizabeth Mehren. 448 pages, New York, 1991, Doubleday. $21.95.

Parenting Mom and Dad: A Guide for the Grown-up Children of Aging Parents. Edited by Michael T. Levy. 267 pages. New York, 1991, Prentice Hall Press. $10.95.

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Recognition, Identification and Prevention of Acute Viral Infections. Edited by Donald M. McLean. 374 pages, illustrated, St. Louis, 1991, Warren Green, Inc. $42.50.

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Tumors of the Placenta and Umbilical Cord. Edited by Douglas R. Shanklin. 203 pages, illustrated. Philadelphia, 1990, B.C. Decker Inc. No price listed.

The Well-Informed Patients' Guide to Caesarean Births. Edited by Kathryn Cox and Judith D. Schwartz. 156 pages. New York, 1990, Dell Publishing. $3.95.

The Well-Informed Patients' Guide to Cataract and Other Eye Surgery. Edited by Mark Speaker and Karyn Feiden. 142 pages. New York, 1990, Dell Publishing. $3.95.

The Well-Informed Patients' Guide to Coronary Bypass Surgery. Edited by Jeffrey Gold and Tony Eprile. 148 pages. New York, 1990, Dell Publishing. $3.95.

The Well-Informed Patients' Guide to Hysterectomy. Edited by Kathryn Cox and Judith Schwartz. 174 pages. New York, 1990, Dell Publishing. $3.95.

Correction

In the article by Harding et al. entitled "A mechanism leading to reduced lung expansion and lung hypoplasia in fetal sheep during oligohydramnios" (Am J Obstet Gynecol 1990;163:1904-1913), in Table I, on page 1907, under *No.*, the first value corresponding to "Fetal tracheal pressure" should read 13 (13) rather than 13 (3).

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, Missouri 63146-3318.

Third Congress of the International Society of Gynecological Endocrinology, Madonna di Campiglio (Italian Alps), February 9-16, 1992. The presentations will relate to the topic: "The Hormones in Gynecological Endocrinology: From Synthesis to Clinical Implications." For further information contact: Prof. Andrea R. Genazzani, President of I.S.G.E., Department of Obstetrics and Gynecology, University of Modena, Via del Pozzo 71, 41100 Modena, Italy. Tel.: 39 59 360255-360224. Fax: 39 59 371401.

Surgical Gynecology, October 18-19, 1991, The Ambassador West Hotel, Chicago, Illinois. For further information contact: Office of Continuing Medical Education, Northwestern University Medical School, 301 East Chicago Ave., Ward 1-004, Chicago, IL 60611-3008. Tel.: (312) 503-8533.

Breastfeeding in the 90s: Issues, Controversies and Solutions, Dr. Ruth Lawrence, keynote speaker, September 12, 1991, St. Louis, Missouri. For information contact: Mary Doerr, RN, St. Mary's Health Center, 6420 Clayton Road, St. Louis, MO 63117. Tel.: (314) 768-8119.

Current Issues in Gynecologic Oncology: HPV and Lower Genital Tract Disease, St. Louis, Missouri, September 7, 1991, sponsored by St. Luke's Hospital Department of Obstetrics and Gynecology. For further information contact: Patty Fogertey, Program Coordinator, Department of Education, St. Luke's Hospital Institute for Health Education, 232 South Woods Mill Road, Chesterfield, MO 63017. Tel.: (314) 542-4762.

Obstetric/Pelvic Imaging, Hands-on Instruction in Diagnostic Ultrasound, Conducted August 12-16, 1991, in Dallas, Texas. For further information call or write Keith Mauney & Associates, Inc., 600 E. Las Colinas Blvd., Suite 250, Irving, TX 75039. Tel.: (214) 402-8250.

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ASPEN
Medical Group

OBSTETRICIAN/GYNECOLOGIST

Aspen Medical Group is seeking additional BC/BE obstetrician/gynecologists to join staff of five. We are an independent, multispecialty group practice with 10 clinics in the Minneapolis/St. Paul metropolitan area.

- Established, challenging practice spanning the full range of obstetrics and gynecology
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For further information, please call or send your curriculum vitae to:

Nancy Borgstrom
Aspen Medical Group
Administration
1020 Bandana Boulevard West
St. Paul, MN 55108
(612) 641-7185

Equal Opportunity Employer.

Excellent opportunity to live and work in beautiful mountainous recreation area with easy access to Pittsburgh and Washington, DC areas. Davis Memorial Hospital, a modern JCAHO facility located seventy (70) miles from tertiary (WVU Hospitals), is actively recruiting a BC/BE OB-GYN wishing to establish a solo practice with coverage by two (2) well trained Board Certified OB-GYNs. Excellent potential for partnership. The practice will serve a population of 90,000. Davis Memorial Hospital is a sole community provider embarking on a major building project to include a new LDRP unit. Lucrative first year guarantee plus new office space beside hospital, staff, malpractice, etc. Elkins offers an excellent quality of life with excellent schools. It is within a one hour drive four major ski resorts. Outstanding outdoor recreational activities such as trout fishing, golf, backpacking, etc., abound. A private four (4) year liberal arts college provides many cultural amenities.

CVs and inquiries to:

James C. Stemple
Assistant CEO
Davis Memorial Hospital
P.O. Box 1484
Elkins, WV 26241
Please call collect 304-637-3151

OBSTETRICIAN/ GYNECOLOGISTS

Group Health Association, one of Washington, D.C.'s most respected names in the health maintenance field, has immediate and future openings for board certified or eligible Obstetrician/ Gynecologists.

Consisting of nine multi-specialty medical centers and 200 physicians, Group Health's highly skilled support staff serves over 150,000 members. This staff attends to the details of clinical administration, allowing you the freedom to devote your energy to practicing medicine. Further, GHA's challenging work environment offers a diverse patient population and affiliation with the area's finest hospitals.

A generous benefits package including vacation, sick leave and insurance coverage (malpractice, life, retirement, disability and dental) awaits you in return for your expertise. In addition, you can look forward to company-paid hospital and licensing fees, professional society dues, continuing education as well as predictable office hours.

If you would like more information, please send your C.V. to the address below or call Mary Hines, Physician Recruiter, on 1-800-336-1442 or in DC Metro area, (202) 364-7442.

Turner Bledsoe, M.D., Medical Director

Group Health Association

4301 Connecticut Avenue, N.W.
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An Equal Opportunity Employer

CHAIR, DEPARTMENT OF OB-GYN

An 830-bed regional tertiary care teaching hospital is seeking a Chair for its Department of OB-GYN. The Allentown Hospital–Lehigh Valley Hospital Center has a fully-approved, free-standing OB-GYN Residency Program with two residents each year and trains a total of 70 residents and 400 medical students.

The hospital is a regional Level III Perinatal Center. Of over 3,000 births in 1990, 42 percent were in the high-risk category. The department is supported by a 20-bed Level III NICU managed by four full-time neonatologists.

In response to the continuing growth of the community and the expansion of the OB-GYN Department, the hospital will ask the new Chair to lead the search for the newly-created position of Vice Chair/Program Director of OB-GYN. The department is also awaiting the arrival of the new Chair to help in the search for a full-time perinatologist. (There are 33 active OB-GYN physicians on staff, including a gynecologic oncologist and reproductive endocrinologist.)

The hospital is a clinical campus of Hahnemann University in Philadelphia, where the new Chair will hold a Vice Chair appointment and an academic appointment at the rank of associate or full professor. The hospital is in Allentown, a burgeoning area in eastern Pennsylvania within close proximity of Philadelphia and New York City.

Candidates must be board certified with administrative experience and familiarity with both academic and community hospital environments. Interested candidates may write or call for information and send curricula vitae, in confidence, to: **Headley S. White, Jr., M.D., Sr. Vice President, Medical and Academic Affairs, c/o HealthSearch, 1243 S. Cedar Crest Blvd., Suite 3000, Allentown, PA 18103, (215) 821-3090.** Descriptive brochure available.

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FACILITIES—METHODIST HOSPITAL OF INDIANA:

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FACULTY POSITIONS
GENERAL OBSTETRICS AND GYNECOLOGY

The University of North Carolina at Chapel Hill, Department of Obstetrics and Gynecology, in conjunction with the Wake Medical Center, Raleigh, North Carolina, and the Wake Area Health Education Center is seeking two board eligible or active candidates in General Obstetrics and Gynecology. One position will require special interest in gynecological surgery and the other special interest in obstetrics.

Wake Medical Center is located in Raleigh, the State Capitol, approximately 40 minutes from Chapel Hill, and is a 560-bed comprehensive tertiary care center with over 4000 deliveries and 1500 surgical procedures each year.

The positions are in the Wake AHEC OB/GYN Department which has four other full-time obstetricians and gynecologists.

Excellent opportunity for clinical research and teaching of the UNC/Chapel Hill Ob/Gyn residents and medical students rotating through Wake Medical Center at all times. Collaboration with the Department of Obstetrics and Gynecology at Chapel Hill is encouraged and anticipated.

Salary and academic rank commensurate with training and experience. Superb fringe benefits package.

Write or contact:

Juan L. Granados, M.D.
Wake AHEC OB/GYN Teaching Director
Wake Medical Center
P.O. Box 14465
Raleigh, NC 27620-4465
(919) 250-8535

The University of North Carolina is an Equal Opportunity/Affirmative Action Employer.

Virginia Baptist Hospital

3300 Rivermont Ave., Lynchburg, VA 24503-2053

Virginia Baptist Hospital is assisting our local staff of obstetricians in recruiting OB/GYN physicians to join established practices. Located in Lynchburg, Virginia at the foot of the Blue Ridge Mountains, Virginia Baptist Hospital is a full-service JCAHO accredited 387-bed hospital serving a population of 250,000. Annually deliveries average 2,400 supported by nine private practicing obstetricians, one perinatologist, and one hospital-based obstetrician with certified nurse midwives providing indigent care services. Virginia Baptist is the designated maternal/child center for this region; therefore, obstetrical practice is confined to this hospital which offers a wide spectrum of birthing options. Lynchburg is convenient to summer and winter resorts and boasts an excellent public school system as well a private schools and five colleges. Salary and benefits are excellent. To arrange a private interview, submit C.V. to Dianne T. Nash, Vice President, Virginia Baptist Hospital, 3300 Rivermont Avenue, Lynchburg, Virginia, 24503, or call (804) 947-4521, collect.

FACULTY POSITION
DIVISION DIRECTOR
MATERNAL-FETAL MEDICINE

- ANTENATAL UNIT
- 5500 DELIVERIES/YEAR
- PREMIER NEWBORN INTENSIVE CARE UNIT
- 7 NEONATOLOGISTS
- 24 HR. IN HOUSE OBSTETRICAL ANESTHESIA
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The Department of Obstetrics & Gynecology at the University of Kansas School of Medicine-Wichita is accepting applications for the position of Director of the Division of Maternal-Fetal Medicine. This represents a unique opportunity to have the best features of both academic medicine and private practice.

Please send curriculum vitae to:

Daniel K. Roberts, M.D., Ph.D.
Professor & Chairman, Dept. of Ob/Gyn
University of Kansas School of Medicine-Wichita
HCA-Wesley Medical Center
550 N. Hillside
Wichita, KS 67214

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PERINATOLOGIST

Outstanding practice opportunity for a BC/BE perinatologist to join an established practice at Iowa's largest private teaching medical center. The hospital, a state designated Level III Perinatal Center, averages over 2,000 deliveries annually and has an active maternal and neonatal transport system. The medical center's Level III Neonatal Intensive Care Unit is staffed by four board certified neonatologists. Complete laboratory facilities are available including cytogenic analysis of both amniotic fluid and trophoblastic tissue. The Antepartum Testing Unit is equipped with computer-based fetal monitors and a color flow Doppler ultrasound unit. Excellent financial and lifestyle opportunities are available to the qualified candidate. Please call 1-800-882-5911 or send CV to Mariann Clark, Medical Search Consultant, 1200 Pleasant Street, Des Moines, Iowa 50309.

OBSTETRICS & GYNECOLOGY

Obstetrician/Gynecologist needed to join a five physician department at a major regional multispecialty clinic in Billings, Montana. Clinic setting includes all specialties and is adjacent to an outstanding tertiary care referral hospital. Excellent opportunity for a top notch board eligible or board certified Obstetrican/Gynecologist looking for a challenging practice in a geographic area offering unlimited recreational potential. Those interested send CV to:

Ann B. Oglesby, M.B.A., M.D.
Medical Director
Billings Clinic
P.O. Box 35100
Billings, Montana 59107-5100

DIRECTOR OF MATERNAL-FETAL MEDICINE

Albert Einstein Medical Center (AEMC), a 600+ bed tertiary care teaching hospital, is seeking a board certified maternal-fetal medicine subspecialist as Director of the Division of Maternal-Fetal Medicine to develop and direct the comprehensive perinatal program in this institution. AEMC is a major referral center in north Philadelphia supporting numerous post-graduate medical education programs, including a fully accredited residency program in obstetrics and gynecology. The Department of Obstetrics and Gynecology has a long-standing tradition of excellence in health-care delivery and supports a variety of clinical research and educational activities. The Division of Maternal-Fetal Medicine serves as a tertiary referral center within the metropolitan Philadelphia area. Its service includes maternal intensive care unit, maternal transport program, high risk obstetrics, genetic counseling, diagnostic genetic procedures (amniocentesis, CVS, cordocentesis), targeted (level II) obstetrical ultrasound and comprehensive antenatal fetal surveillance. Our Department is supported by a Level III NICU and a Cytogenetic Laboratory. There is a strong institutional support and emphasis in research with funds and laboratory space available for basic research. A construction project is under way for a new labor and delivery suite and a comprehensive fetal diagnostic center. We are also planning to develop a maternal-fetal medicine fellowship training program with Temple University.

AEMC is a major affiliate of Temple University. Academic appointment is available for all full-time AEMC faculty. Highly competitive salary and very attractive fringe benefits are included. If you are interested in this position or would like additional information about this unique opportunity, please write to *Sze-ya Yeh, M.D., Chairman, Department of Obstetrics and Gynecology, Albert Einstein Medical Center, 5401 Old York Road, Klein #410, Philadelphia, PA 19141.* Telephone **(215) 456-6993,** FAX **(215) 456-6271.**

DIRECTOR, DIVISION OF MATERNAL FETAL MEDICINE

Director, Division of Maternal Fetal medicine at university medical center affiliate of Tufts University School of Medicine. This challenging position provides opportunity to lead Maternal Fetal Medicine Division of the Department of OB/GYN of St. Margaret's Hospital for Women and St. Elizabeth's Hospital of Boston at SEH campus. Successful candidate must qualify for senior level faculty appointment at Tufts, have experience in leadership of OB/GYN and Maternal Fetal Medicine residency education and research; and interest in medical student education. Competitive salary and benefits package available. Please forward curriculum vitae to:

James P. Durkan, M.D.
Chairman, Department of OB/GYN
St. Elizabeth's Hospital of Boston
736 Cambridge St.
Brighton, MA 02135

COLORADO

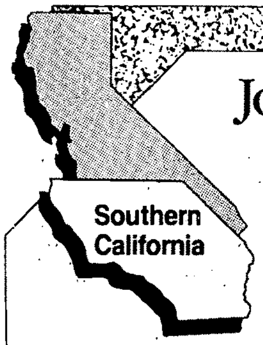
BC/BE female/male DO/MD sought to join progressive 2 man group. Association leading to early partnership with excellent income potential. Office located in one of Denver's fastest growing north west suburbs. Opportunity available immediately. Send inquiries and C.V. to:

A. E. Murray
Director, Medical Staff Services
8300 West 38th Avenue
Wheat Ridge, Colorado 80033

Obstetrician/ Gynecologists

Marshfield Clinic, a 350 physician multispecialty group practice is seeking BE/BC Obstsetrician/Gynecologists to join expanding regional referral centers in Chippewa Falls and Woodruff/Minocqua, Wisconsin. These are beautiful four-season recreational locations combining professional excellence with a family-oriented life style. Both have newly constructed Clinics with comparably modern hospitals. These practices provide an opportunity to enjoy the autonomy of a private practice with the financial and professional resources of a major multispecialty group. **Chippewa Falls** is located 1 1/2 hours from downtown Minneapolis/St. Paul and borders Eau Claire, Wisconsin, a city of nearly 80,000 which includes a major 4 year campus of the University of Wisconsin. Known as the "Lakeland Area," **Woodruff/Minocqua** is truly one of the most attractive vacation areas in the upper Midwest. An emphasis on quality practice and life style is combined with a very competitive salary and one of the finest fringe benefit packages in the country. For additional information, please send C.V. and references to: **David L. Draves, Director Regional Development, Marshfield Clinic**, 1000 North Oak Avenue, Marshfield, WI 54449, or call 1-800-826-2345, extension 5376.

MARSHFIELD CLINIC



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Send your curriculum vitae to **Irwin P. Goldstein, M.D., Associate Medical Director, SCPMG, Dept. 006, Walnut Center, Pasadena, CA 91188-8013.**

Or call: **1-800-541-7946**

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OBSTETRICIAN/GYNECOLOGIST

Are you looking for a practice opportunity that offers unlimited potential and rewards? If you are, consider the options available with one of northwest Ohio's leading health care systems. We currently have several exciting practice opportunities available for board certified/board eligible OB/GYN's including solo, partnership, and group practices. Obstetrical referrals are also available from physicians who recently limited their practice to gynecology. For those physicians interested in teaching to augment their practice, precepting opportunities are also available in conjunction with one of our hospital-based family practice residency programs.

These opportunities offer an exceptional support package which includes a generous guaranteed salary, malpractice insurance, family health insurance and disability insurance, overhead expenses, moving expenses, and much more!

This area of the Great Lakes region offers a host of social, cultural, recreational, and sporting activities including several college and professional teams within a 50-mile radius.

For further details about these exciting practice opportunities, please send your curriculum vitae to: P.O. Box 2450, Toledo, Ohio 43606.

OB/GYN

A 180-bed County Hospital located in the San Francisco Bay Area and serving a Medi-Cal and HMO population is seeking a BE/BC OB/GYN. Duties will include active GYN surgery service and outpatient GYN and Prenatal Clinics; supervision of U.C. Davis-affiliated residents in a 150 delivery/month OB service. Night call will be shared with four others and can be taken from home. Competitive salary and benefits. Send C.V. to: **D. Kent Hobert, M.D., Department OB/GYN, Merrithew Memorial Hospital, 2500 Alhambra Avenue, Martinez, CA 94553.**

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AND CLINICS

OB/ GYN

Staff Physician for Midwest Infant Mortality Project. Seeking a BC/BE graduate with an interest in prenatal health. New and renovated program sites receive support from the staff of one of the largest hospitals in the Midwest. Hospital staff rank and privileges. Teaching responsibilities.

Medical Director/Clinician for freestanding newly renovated unit in a Midwestern community of 50,000. Prefer BC/BE residency senior or graduate with an interest in prenatal health programs. Serve with a dedicated staff.

Both are staff positions. Packages include malpractice and benefits. To learn more about these positions, please call or send CV to:

Marion Mason
Mason & Associates, P.O. Box 12558
Fort Wayne, Indiana 46863-2558
(219) 484-2007 • FAX (219) 484-8655
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OB/GYN WISCONSIN

Multi-specialty group in suburban Milwaukee seeks BC/BE Obstetrician/Gynecologist to join 8 physician Ob/Gyn department. Single site, single hospital practice. Competitive salary and fringe benefits.

Forward Inquiries and CV to:

Administrator
P.O. Box 427
Menomonee Falls, WI
53052-0427

OBSTETRICIAN / GYNECOLOGIST

At the request of a local obstetrician/gynecologist located in Peoria, Illinois, we are seeking an additional OB/GYN physician to join this rapidly expanding private practice. This is an opportunity to associate with a university affiliated teaching hospital.

Methodist is a 530-bed teaching hospital affiliated with the University of Illinois College of Medicine at Peoria. All specialties are represented on our Active Staff. Methodist possesses two MRIs, an Outpatient Diagnostic Unit, a new seven-story Physicians' Plaza, and a PET scanner which was installed earlier this year and is now operational.

Peoria, an All-American city with a population of over 120,000 and a referral base of 350,000, is located on the Illinois River in scenic Central Illinois. There are numerous sports, recreational, and cultural activities available locally with the advantages of Chicago and St. Louis less than three hours away.

Excellent compensation and benefit packages are being offered. If interested, please submit your CV to:

Harvey Lightbody
Vice-President of Physician Recruitment
1-800-621-8543
METHODIST MEDICAL CENTER OF ILLINOIS
221 N.E. Glen Oak Avenue
Peoria, Illinois 61636
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Obstetrics/ Gynecology

The joining of two proud traditions in Chicago means you can now practice medicine the way you've always wanted.

Benefit from the combined strengths of MICHAEL REESE'S century-long history of providing the highest quality care and services and HUMANA'S expanding national organizational expertise and fiscal/management resources.

You'll practice in the collegial environment of a multi-specialty group setting with continuous access to premier teaching and research opportunities and innovative medical technologies.

Our growing network of 21 health centers and 17 affiliated hospitals, conveniently located throughout the metropolitan area, offers you the flexibility to choose a schedule that accommodates your professional and lifestyle needs at an income level you set.

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2545 King Drive
Chicago 60616
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Affiliated with Harvard Medical School (clinical appointment available). We invite applications from board eligible obstetrician/gynecologists interested in primary ob/gyn care, working with midwives, and teaching. New obstetrical unit and Level II nursery. Boston area: offering excellent schools, housing, recreation, and cultural events. Competitive salary and benefits. Equal opportunity employer.

Send c.v. to:

Martin B. Wingate, M.D., Chairman
Department of Obstetrics
and Gynecology
Mount Auburn Hospital
330 Mount Auburn Street
Cambridge, MA 02238
(617) 499-5161

OB/GYN
HOUSTON AREA

118-bed hospital just east of Houston, Texas has an opportunity for an OB/GYN. Fantastic practice potential and access to the coast of Texas makes this a premier opening. Hospital will subsidize physician's net income. Send CV or call:

Jeffery T. Lawrence
JACKSON AND
COKER, INC.
115 Perimeter
Center Place
Suite 380 11004
Atlanta, GA 30346
Tel. 1-800-544-1987

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CHIEF, OBSTETRICS/GYNECOLOGY

Newton-Wellesley Hospital, a 340-bed Tufts affiliated community teaching hospital, located less than 10 miles west of Boston, is conducting a search for a Chief to head the Department of Obstetrics & Gynecology. This position is full-time including practice opportunities.

Candidates must be board certified in Obstetrics and Gynecology and have strong clinical interests and skills, a commitment to family-centered patient care, management skills, and an ability to lead an active educational program geared to the needs of a rapidly growing department. Interest and expertise in one of the subspecialties of Obstetrics and Gynecology is preferred.

There are currently 3600 deliveries per year at the Newton-Wellesley Hospital, with continued growth anticipated. The Maternity Facility consists of a 20-bed LDRP Unit, 6 LDR suites, 2 C-section/high risk delivery suites, 2-bed PACU and 24-bed post partum unit, and an antenatal testing unit. This service is supported by a 12-bed, Level II, special care nursery, as well as full-time neonatology and anesthesia coverage.

The successful candidate will be responsible for the ongoing clinical, administrative, and teaching activities for the department, as well as for the direction of its active growth and development.

Interested candidates should forward their curriculum vitae to: Joel Rubenstein, M.D., Chairman, Search Committee, Newton-Wellesley Hospital, 2000 Washington Street, Newton, MA 02162.

NWH is an equal opportunity employer.

Newton-Wellesley
Hospital

Oakwood Hospital

Department of Obstetrics and Gynecology Resident Education Program Director

We seek a Program Director for our fully accredited, independent, 16 person, Resident Education Program in Obstetrics and Gynecology. Nine full-time faculty members. Three well established divisions directed by full-time, certified, sub-specialists. Annually there are 4,500 deliveries and 3,500 gynecologic surgical procedures at our 615-bed, acute tertiary care hospital.

Interested candidates should send their curriculum vitae to: **Martin Farber, M.D., Senior Vice President, OAKWOOD HOSPITAL, 18101 Oakwood Boulevard, P.O. Box 2500, Dearborn, MI 48123-2500, or phone (313) 593-7803** for further information. Equal Opportunity Employer.

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Private Practice

Obstetrician Gynecologist

Private practice opportunity for BC/BE Obstetrician/Gynecologist to join or affiliate with a six member Board Certified OB/GYN group in Sioux Falls, South Dakota.

OB/GYN, LTD. has a state of the art office with advanced ultrasound and monitoring facilities, laboratory and mammography on the premises. Located adjacent to a highly regarded 476 bed tertiary care hospital which features a 37 bed Level III NICU with full-time neonatal coverage.

Sioux Falls is an attractive city with a population of 100,000 plus. Our community has a strong economic base, growing population and a progressive educational system including two local colleges. Medical School appointment possible.

Excellent salary, fringe benefits and incentives.

Please send curriculum vitae in confidence to:
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Sioux Falls, South Dakota 57105
Please call:
1-800-468-3333 for informational packet.

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Physician leader to provide gynecology and general medical services for students and to supervise nurses and mid-level practitioners in a growing, innovative women's health department. M.D. or D.O., BC/BE in ob/gyn, family practice or internal medicine with 4-5 years' previous experience in adolescent/young adult medicine.

This position offers competitive salary with excellent benefits package, malpractice insurance paid by the University. Located in central Pennsylvania in picturesque Happy Valley.

Send letter of application, resume and salary requirements to:

Employment Division
JOB #: AJO-1590
120 South Burrowes Street
University Park, PA 16801
APPLICATION DEADLINE: SEPTEMBER 15, 1991

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Finger Lakes Region
Ithaca, New York

Unique opportunity for patient oriented physician in comprehensive OB-GYN service, building on established patient base of 6,000 patients. To include out-patient gynecology, obstetrics, surgery, infertility, and abortions. First call protection from nurse-midwives. Progressive university community (home of Cornell University) offers cultural attractions of a city with the benefits of small town living. Competitive salary, excellent benefits including malpractice. Summer/Fall 1991.

Send CV To: Joyce Leslie, M.D., Planned Parenthood of Tompkins County, 314 W. State St., Ithaca, NY 14850.

Planned Parenthood®
of Tompkins County

THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
600 Highland Avenue, H4/654 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/Gynecology
University of Wisconsin Medical School
Sinai Samaritan Medical Center
2000 West Kilbourn Avenue
Milwaukee, WI 53233
(414) 937-5600

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PERINATAL ASSOCIATES OF NORTHERN CALIFORNIA

Are you a clinical perinatologist? There is still time to join four perinatologists and one geneticist-OB/GYN looking for one more perinatologist who: desires superior environment for patient care, in a private, modern, level 3 facility doing 6,000 deliveries a year with superior perinatal outcome; likes personal, direct hands-on patient care; needs PUBS, IUTxF, CVS and complicated pregnancies, level 2 ultrasound; demands partners, not superiors; wants to live in clean, warm, not humid, snow-free (but 60 minutes from skiing) Sacramento, California; feels one's family has priorities, and is committed to the private practice of medicine. For more information, call (916) 733-1755, extension 2773.

Hanns C. Haesslein, M.D.
Perinatal Associates
of Northern California
5301 F Street
Sacramento, California 95819

NORTHERN CALIFORNIA

The Permanente Medical Group, a growing multispecialty group, is recruiting physicians in a variety of specialties for positions throughout our beautiful Northern California region: the San Francisco Bay Area, Sacramento and the Central Valley.

Obstetrician/Gynecologist

BC/BE Obstetrician/Gynecologist sought to join small congenial clinic setting within established pre-paid multispecialty group. Practice consists of general obstetrics and gynecology, high risk obstetrics and infertility. Expertise in colposcopy, laparoscopy, hysteroscopy and laser are welcomed. Association with resident staff and teaching opportunities available.

Kaiser Permanente offers competitive salaries and excellent benefits including full coverage malpractice insurance, medical, dental and group life insurance, vacation, educational leave and an excellent retirement program.

For more information, send CV to: Victor David, M.D., Chief, Obstetrics and Gynecology, The Permanente Medical Group, 901 Nevin Avenue, Richmond, CA 94801. EOE

KAISER PERMANENTE
Good People. Good Medicine.

St. Louis, Missouri
Chief of Service/OB-GYN

Board Eligible/Board Certified OB/GYN needed to join this 15 member group of primary care physicians. You will develop and implement policy and procedures in women's health/family planning. This is a wonderful career opportunity.

♀ No deliveries. No on-call or weekend responsibility.
♀ Newer facility with lab, x-ray, mammography, and pharmacy on site.
♀ All visits are scheduled by appointment, averaging 20 patients per day.
♀ Clinic hours are 8:00 am to 4:30 pm, Monday-Friday.
♀ Reimbursement for malpractice insurance.
♀ Fees based on qualifications and experience.

For complete details, contact:
Mallarry Dierkes
Spectrum Emergency Care, Inc.
PO Box 27352
St. Louis, MO 63141
1-800-325-3982, ext. 1029

State University of New York
Health Science Center
Syracuse

MATERNAL-FETAL MEDICINE SUBSPECIALIST
ASSISTANT PROFESSOR/ ASSOCIATE PROFESSOR

The Department of Obstetrics and Gynecology of the SUNY Health Science Center at Syracuse is actively recruiting a full-time Perinatologist for its Division of Maternal-Fetal Medicine.

The teaching hospital serves as the only Tertiary Care Center for the Regional Perinatal Program encompassing the 15 counties of Central New York with a population of 2.3 million.

Excellent opportunity to participate in teaching in a University based setting and collaborate in ongoing research in clinical Perinatology, Ultrasound, and Prenatal Diagnosis.

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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
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is seeking applicants for four (4) full-time faculty positions for a new women's health center and academic program at its Milwaukee Clinical Campus.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department supports a residency program, the largest perinatal service in Wisconsin (5500 deliveries), consultative ultrasound, genetic and infectious disease programs, and reproductive/endocrine services (including an IVF program). The department is affiliated with Planned Parenthood of Wisconsin and St. Luke's Medical Center in Milwaukee.

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Chairman
Department of Obstetrics/
Gynecology
University of Wisconsin
Medical School
600 Highland Avenue, H4/564 CSC
Madison, WI 53792

Fredrik F. Broekhuizen, M.D.
Chairman and Chief
Milwaukee Campus
Department of Obstetrics/
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THE DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
UNIVERSITY OF WISCONSIN MEDICAL SCHOOL

is seeking applicants for two (2) additional full-time faculty positions in MATERNAL FETAL MEDICINE for a new women's health center and academic program at its Milwaukee Clinical Campus. The current faculty includes two perinatologists.

Sinai Samaritan Medical Center has recently consolidated its obstetrical and gynecological services in a redesigned facility in downtown Milwaukee. The department has the largest perinatal service in Wisconsin (5500 deliveries) and has a dedicated antenatal testing and perinatal ultrasonography unit, a midwifery, and adolescent pregnancy service. The department has an affiliation with St. Luke's Medical Center in Milwaukee for its residency program.

Qualifications include board candidacy or certification in maternal-fetal medicine and a commitment to teaching residents, medical students, and clinical research. Competitive salary package. Contact:

Chester B. Martin, M.D.
Chairman
Department of Obstetrics/
Gynecology
University of Wisconsin
Medical School
600 Highland Avenue,
H4/654 CSC
Madison, WI 53792

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ICATIONS AND USAGE: PREVENTION OF PREGNANCY.

NTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following ditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophle- s or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected cinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-depen- t neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice h prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

igarette smoking increases the risk of serious cardiovascular side effects from oral contracep- ive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and s quite marked in women over 35 years of age. Women who use oral contraceptives should be trongly advised not to smoke.

use of oral contraceptives is associated with increased risks of several serious conditions including ocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the of serious morbidity or mortality is very small in healthy women without underlying risk factors. The of morbidity and mortality increases significantly in the presence of other underlying risk factors such hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives uld be familiar with the following information relating to these risks. The information contained in this f summary is principally based on studies carried out in patients who used oral contraceptives with her formulations of estrogens and progestogens than those in common use today. The effect of long m use of the oral contraceptives with lower formulations of both estrogens and progestogens remains be determined. Throughout this brief summary epidemiological studies reported are of two types: ospective or case control studies and prospective or cohort studies. Case control studies provide a asure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contra- tive users to that among nonusers. The relative risk does not provide information on the actual clinical urrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in incidence of disease between oral contraceptive users and nonusers. The attributable risk does pro- e information about the actual occurrence of a disease in the population. For further information, the der is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER SCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been ociated with oral contraceptive use. This risk is primarily in smokers or women with other underlying factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and etes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has n shown to contribute substantially to the incidence of myocardial infarctions in women in their -thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated h circulatory disease have been shown to increase substantially in smokers, especially in those 35 rs of age and older among women who use oral contraceptives. Oral contraceptives may compound the cts of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In ticular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, le estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase od pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been ociated with an increased risk of heart disease. Oral contraceptives must be used with caution in nen with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboem- c and thrombotic disease associated with the use of oral contraceptives is well established. Case trol studies have found the relative risk of users compared to non-users to be 3 for the first episode of erficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for nen with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the tive risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospital- ion. The risk of thromboembolic disease associated with oral contraceptives is not related to length of and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative mboembolic complications has been reported with the use of oral contraceptives. The relative risk of ous thrombosis in women who have predisposing conditions is twice that of women without such lical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and ing and following prolonged immobilization. Since the immediate postpartum period is also associated an increased risk of thromboembolism, oral contraceptives should be started no earlier than four ks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contracep- s have been shown to increase both the relative and attributable risks of cerebrovascular events ombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), ertensive women who also smoke. Hypertension was found to be a risk factor for both users and -users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for rs with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non- kers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for kers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hyper- sion. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from contraceptives. A positive association has been observed between the amount of estrogen and gestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density proteins (HDL) has been reported with many progestational agents. A decline in serum high density proteins has been associated with an increased incidence of ischemic heart disease. Because estro- s increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved ween doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. activity and amount of both hormones should be considered in the choice of an oral contraceptive. imizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For particular estrogen/progestogen combination, the dosage regimen prescribed should be one which tains the least amount of estrogen and progestogen that is compatible with a low failure rate and the ds of the individual patient. New acceptors of oral contraceptive agents should be started on prepara- s containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two lies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a ly in the United States, the risk of developing myocardial infarction after discontinuing oral contracep- s persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or e years, but this increased risk was not demonstrated in other age groups. In another study in Great ain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation ral contraceptives, although excess risk was very small. However, both studies were performed with contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF RTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have mated the mortality rate associated with different methods of contraception at different ages. These mates include the combined risk of death associated with contraceptive methods plus the risk attribu- e to pregnancy in the event of method failure. Each method of contraception has its specific benefits risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke 40 and older who do not smoke, mortality associated with all methods of birth control is low and w that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who oral contraceptives have a significant increase in mortality higher than those using other methods of control. These facts must be weighed in conjunction with failure rates for other methods and the risk ciated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous emiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical er in women using oral contraceptives. While there are conflicting reports most studies suggest that of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. e studies have reported an increased relative risk of developing breast cancer, particularly at a ger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.** **ADVERSE REACTIONS:** An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis. **OVERDOSAGE:** Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

Revised 9/89

ORTHO

ORTHO PHARMACEUTICAL CORPORATION
Raritan, New Jersey 08869

Outstanding Clinical Performance Reflected Inside & Outside

13 DEC 1991

INSIDE

Low Metabolic Impact is reflected inside your ORTHO-NOVUM[*] 7/7/7 patients[†]: No clinically significant change in plasma glucose and insulin levels in nondiabetic women.[‡,1] Mean values for total cholesterol, HDL or LDL, and blood pressure also show no significant change.[§]

Excellent Cycle Control is another reflection of outstanding performance with ORTHO-NOVUM 7/7/7. You can expect low discontinuation rates due to breakthrough bleeding, spotting, and amenorrhea.

OUTSIDE

Low Incidence of Androgenic Side Effects—such as acne, oily skin, or hirsutism—is a visible reflection that your patients may notice; another is no clinically significant change in mean body weight. Inside and outside, the outstanding performance of ORTHO-NOVUM 7/7/7 is also an excellent reflection on you.

Low incidence of androgenic side effects

21- and 28-day regimens

ORTHO-NOVUM 7/7/7 Tablets

TRADEMARK

norethindrone/ethinyl estradiol

ORTHO PHARMACEUTICAL
CORPORATION
Raritan, New Jersey 08869

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[†] Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

[‡] Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

[§] An increase in blood pressure has been reported in women taking oral contraceptives.

[1] Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med*. 1989;34(8):540-542.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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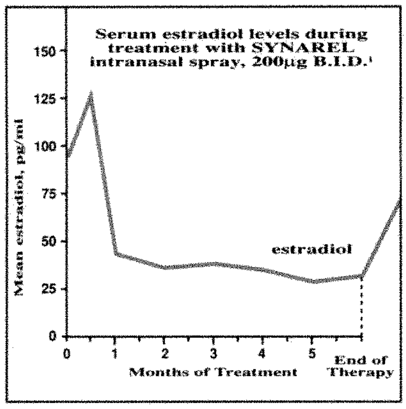
IN THE MANAGEMENT
OF ENDOMETRIOSIS

enough is enough

Insufficient estradiol suppression may not resolve the symptoms of endometriosis. Excessive suppression may create additional problems for your patients.
SYNAREL intranasal spray promises no less than you need – and no more than you want.

Power to suppress estradiol to levels that result in effective relief

SYNAREL intranasal spray, 200μg B.I.D., lowers serum estradiol to near menopausal levels within approximately 1 month—and maintains those levels throughout the 6-month course of therapy.* As a result, 92% of patients (n=143) taking SYNAREL were symptom-free or had only mild symptoms following a 6-month course of therapy. And, of the 60% of patients (n=110) who had complete relief of symptoms, 83% remained symptom-free or experienced only mild symptoms 6 months after treatment with SYNAREL was discontinued. Retreatment cannot be recommended since safety data for retreatment are not currently available.



Control in the management of hypoestrogenic and therapeutic effects

SYNAREL produces minimal impact on bone, as well as a predictable and manageable hypoestrogenic profile. What's more, SYNAREL lets you terminate treatment immediately should that be required for any reason—unlike the depot form of GnRH[†] therapy. In fact, drug clearance with SYNAREL usually occurs within 24 hours.[2]

Bone density changes during and after treatment with SYNAREL intranasal spray, 400 μg/day [Percent changes from baseline]

Site Method Units	Time of Study Baseline	End of Treatment	Post-treatment
Lumbar Vertebrae QCT* Integral (g/vertebrae) n=21	13.4	12.8 –4.3%	13.1 –3.3%
Trabecular Bone of Vertebrae QCT* (mg/cc) n=23	161.3	147.6 –8.7%	152.0 –4.9%
Lumbar Vertebrae DPA† (g/cm²) n=6	0.98	0.93 –5.9%	0.96 –1.4%
Distal Radius SPA‡ (Units) n=6	40.2	39.5 –1.6%	39.6 –0.8%

*Quantitative computed tomography ‡Single photon absorptiometry
†Dual photon absorptiometry

SYNAREL is also well tolerated, with a low incidence of nasal irritation (10%). And, since SYNAREL lowers estrogen without adding androgens, it helps you avoid potentially irreversible androgenic effects. (The most frequent side effects are those associated with hypoestrogenism.)

In the management of endometriosis, only SYNAREL can balance the power you need with the control you want in a unique intranasal spray. For more information, please call 1-800-727-0400.

*Pregnancy should be ruled out before therapy with SYNAREL is initiated, and patients should be advised to use a nonhormonal method of contraception when taking SYNAREL.
†Gonadotropin-releasing hormone analog.
[1] Syntex document #829-265-A.
[2] Syntex document #829-265-B.
Please see brief summary of prescribing information on next page.

SYNAREL®

(NAFARELIN ACETATE)

NASAL SOLUTION, 2 MG/ML

Setting The Pace In Endometriosis Therapy

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Committed to the OB/GYN and women's healthcare

BRIEF SUMMARY

SYNAREL® Nasal Solution contains nafarelin acetate (2 mg/mL, expressed as nafarelin base), benzalkonium chloride, glacial acetic acid, sodium hydroxide or hydrochloric acid, sorbitol, and purified water.
INDICATIONS AND USAGE: For management of endometriosis. Experience is limited to women 18 years and older treated for 6 months.
CONTRAINDICATIONS: Hypersensitivity to GnRH, GnRH agonist analogs or any excipients in SYNAREL; undiagnosed abnormal vaginal bleeding; pregnancy or possibility of pregnancy while taking the drug because SYNAREL may cause fetal harm when administered to a pregnant woman. If SYNAREL is used during pregnancy or if the patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus; use in women who are breast feeding.
WARNINGS: Safe use in pregnancy has not been established clinically. Before starting treatment pregnancy must be excluded.
When used regularly at the recommended dose, SYNAREL usually inhibits ovulation and stops menstruation. Contraception is not insured, particularly if patients miss successive doses. Therefore, patients should use nonhormonal contraception. Advise patients to see their physician if they believe they may be pregnant. If a patient becomes pregnant during treatment, discontinue the drug; apprise patient of the potential risk to the fetus.
PRECAUTIONS General: Ovarian cysts have been reported in the first 2 months of therapy. These may resolve spontaneously or may require drug discontinuation or surgery.
Information for Patients: 1. Since menstruation should stop with SYNAREL, the patient should notify her physician if regular menstruation persists. If patient is compliant and menstruation persists to second month, consider doubling the dose. Counsel noncompliant patients on importance of taking SYNAREL regularly. 2. Patients should not use SYNAREL if pregnant, breast feeding, have undiagnosed abnormal vaginal bleeding, or are allergic to any of its ingredients. 3. Safe use in pregnancy has not been established. Therefore, a nonhormonal method of contraception should be used during treatment. If a patient becomes pregnant during treatment, she should discontinue the drug and consult her physician. 4. Adverse events occurring most frequently in clinical studies are associated with hypoestrogenism. Nasal irritation occurred in about 10% of patients. 5. Induced hypoestrogenic state results in a small loss in bone density over a 6-month treatment course. Some of this loss may not be reversible. During one six-month treatment period, this should not be important. In patients with major risk factors for decreased bone mineral content, i.e., chronic alcohol and/or tobacco use, strong family history of osteoporosis, or chronic use of drugs that can reduce bone mass such as anticonvulsants or corticosteroids, SYNAREL may pose additional risk. In these patients risks and benefits must be weighed carefully. Repeated courses of treatment with GnRH analogs are not advisable in patients with major risk factors for loss of bone mineral content. 6. Patients with intercurrent rhinitis should consult their physician for use of a topical nasal decongestant. If a topical nasal decongestant is required during treatment, it must be used at least 30 minutes after SYNAREL dosing to decrease the possibility of reducing drug absorption. 7. Retreatment cannot be recommended since safety data beyond 6 months are not available.
Drug Interactions: No pharmacokinetic-based drug-drug interaction studies have been conducted. Nafarelin acetate is a peptide primarily degraded by peptidase and not by cytochrome P-450 enzymes, and is only about 80% bound to plasma proteins at 4°C; therefore drug interactions would not be expected to occur.
Drug/Laboratory Test Interactions: SYNAREL in therapeutic doses suppresses the pituitary-gonadal system. Normal function is usually restored 4-8 weeks after treatment is discontinued. Therefore, diagnostic tests of pituitary gonadotropic and gonadal functions during treatment and up to 4-8 weeks after discontinuation of SYNAREL may be misleading.
Carcinogenesis, Mutagenesis, Impairment of Fertility: Carcinogenicity studies were conducted in rats and mice at intramuscular doses up to 110 times and 560 times the maximum recommended human intranasal dose, respectively. These multiples of the human dose are based on the relative bioavailability of the drug by the two routes of administration. As with other GnRH agonists, nafarelin acetate given to laboratory rodents at high doses for prolonged periods induced hyperplasia and/or neoplasia of endocrine organs. At 24 months, there was an increase in the incidence of pituitary tumors (adenoma/carcinoma) in high-dose female rats and a dose-related increase in male rats. There was an increase in pancreatic islet cell adenomas in both sexes, and in benign testicular and ovarian tumors in the treated groups. There was a dose-related increase in benign adrenal medullary tumors in treated female rats. In mice, there was a dose-related increase in Harderian gland tumors in males and an increase in pituitary adenomas in high-dose females. No metastases of these tumors were observed. It is known that tumorigenicity in rodents is particularly sensitive to hormonal stimulation.

Mutagenicity studies using bacterial, yeast, and mammalian systems provided no evidence of mutagenic potential.

Reproduction studies in male and female rats have shown full reversibility of fertility suppression when treatment was discontinued after continuous administration for up to 6 months.
Pregnancy, Teratogenic Effects: *Pregnancy Category X.* Intramuscular SYNAREL was administered to rats throughout gestation at about 0.5, 2, and 7 times the maximum recommended human intranasal dose based on the relative bioavailability by the two routes of administration. An increase in major fetal abnormalities was seen in 4/80 fetuses at the highest dose. A similar study at the same doses in rats and studies in mice and rabbits at doses up to 600 μg/kg/day and 0.18 μg/kg/day, respectively, failed to demonstrate an increase in fetal abnormalities after administration throughout gestation. In rats and rabbits, there was a dose-related increase in fetal mortality and a decrease in fetal weight with the highest dose.
Nursing Mothers: It is not known whether SYNAREL is excreted in human milk. Because many drugs are excreted in human milk, and because the effects of SYNAREL on lactation and/or the breastfed child have not been determined, SYNAREL should not be used by nursing mothers.
Pediatric Use: Safety and effectiveness in children have not been established.
ADVERSE REACTIONS: The most frequently reported adverse reactions were those related to hypoestrogenism. In controlled studies comparing SYNAREL (400 μg/day) and danazol (600 or 800 mg/day), the following adverse reactions were most frequently reported and thought to be drug-related.
Hypoestrogenic: hot flashes (SYNAREL 90%, danazol 69%), decreased libido (22%, 7%), vaginal dryness (19%, 7%), headaches (19%, 21%), emotional lability (15%, 18%), insomnia (8%, 4%); **Androgenic:** acne (13%, 20%), myalgia (10%, 23%), reduced breast size (10%, 16%), edema (8%, 23%), seborrhea (8%, 17%), weight gain (8%, 28%), hirsutism (2%, 6%), increased libido (1%, 6%); **Local:** nasal irritation (10%, 3%); **Miscellaneous:** depression (2%, 5%), weight loss (1%, 3%).

Less than 1% of patients experienced paresthesia, palpitations, chloasma, maculopapular rash, eye pain, urticaria, asthenia, lactation, breast engorgement, and arthralgia. In clinical trials, immediate hypersensitivity thought to be possibly or probably related to nafarelin occurred in 3 (0.2%) of 1509 healthy subjects or patients.
Changes in Bone Density: After 6 months' treatment vertebral trabecular bone density and total vertebral bone mass, measured by quantitative computed tomography (QCT), decreased by an average of 8.7% and 4.3%, respectively, compared to pretreatment levels. There was partial recovery of bone density post-treatment; the average trabecular bone density and total bone mass were 4.9% and 3.3% less than pretreatment levels, respectively. Total vertebral bone mass, measured by dual photon absorptiometry (DPA), decreased by a mean of 5.9% at end of treatment. Mean total vertebral mass, re-examined by DPA 6 months post-treatment, was 1.4% below pretreatment levels. There was little, if any, decrease in the mineral content in compact bone of the distal radius and second metacarpal. Use for longer than the recommended 6 months or in presence of other known risk factors for decreased bone mineral content may cause additional bone loss.
Changes in Laboratory Values: *Plasma enzymes.* During clinical trials SGOT and SGPT levels were more than twice the upper limit of normal in only one patient each. There was no other evidence of abnormal liver function and levels returned to normal after treatment was stopped.
Lipids. At enrollment and at end of treatment, 9% of patients on SYNAREL 400 μg/day and 2% on danazol had total cholesterol values above 250 mg/dL.

Of those with pretreatment cholesterol values below 250 mg/dL, 6% on SYNAREL and 18% on danazol had post-treatment values above 250 mg/dL.

Mean (± SEM) pretreatment values for total cholesterol from all patients were 191.8 (4.3) mg/dL in the SYNAREL group and 193.1 (4.6) mg/dL in the danazol group. At the end of treatment, mean values from all patients were 204.5 (4.8) mg/dL in the SYNAREL group and 207.7 (5.1) mg/dL in the danazol group. These increases from pretreatment values were significant ($p < 0.05$) in both groups.

Triglycerides were increased above the upper limit of 150 mg/dL in 12% of the patients on SYNAREL and in 7% of the patients on danazol.

At the end of treatment, no patients on SYNAREL had abnormally low HDL cholesterol fractions (< 30 mg/dL) compared with 43% of patients on danazol. No patients on SYNAREL had abnormally high LDL cholesterol fractions (> 190 mg/dL) compared with 15% of those on danazol. There was no increase in the LDL/HDL ratio in patients on SYNAREL, but approximately a 2-fold increase in the LDL/HDL ratio in patients on danazol.
Other changes. In comparative studies, the following changes were seen in approximately 10% to 15% of patients. SYNAREL was associated with elevations of plasma phosphorous and eosinophil counts, and decreases in serum calcium and WBC counts. Danazol therapy was associated with an increase of hematocrit and WBC.
OVERDOSAGE: At present there is no clinical evidence of adverse effects following overdosage of GnRH analogs.

SYNTEX

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PALO ALTO, CA 94304

April 1991
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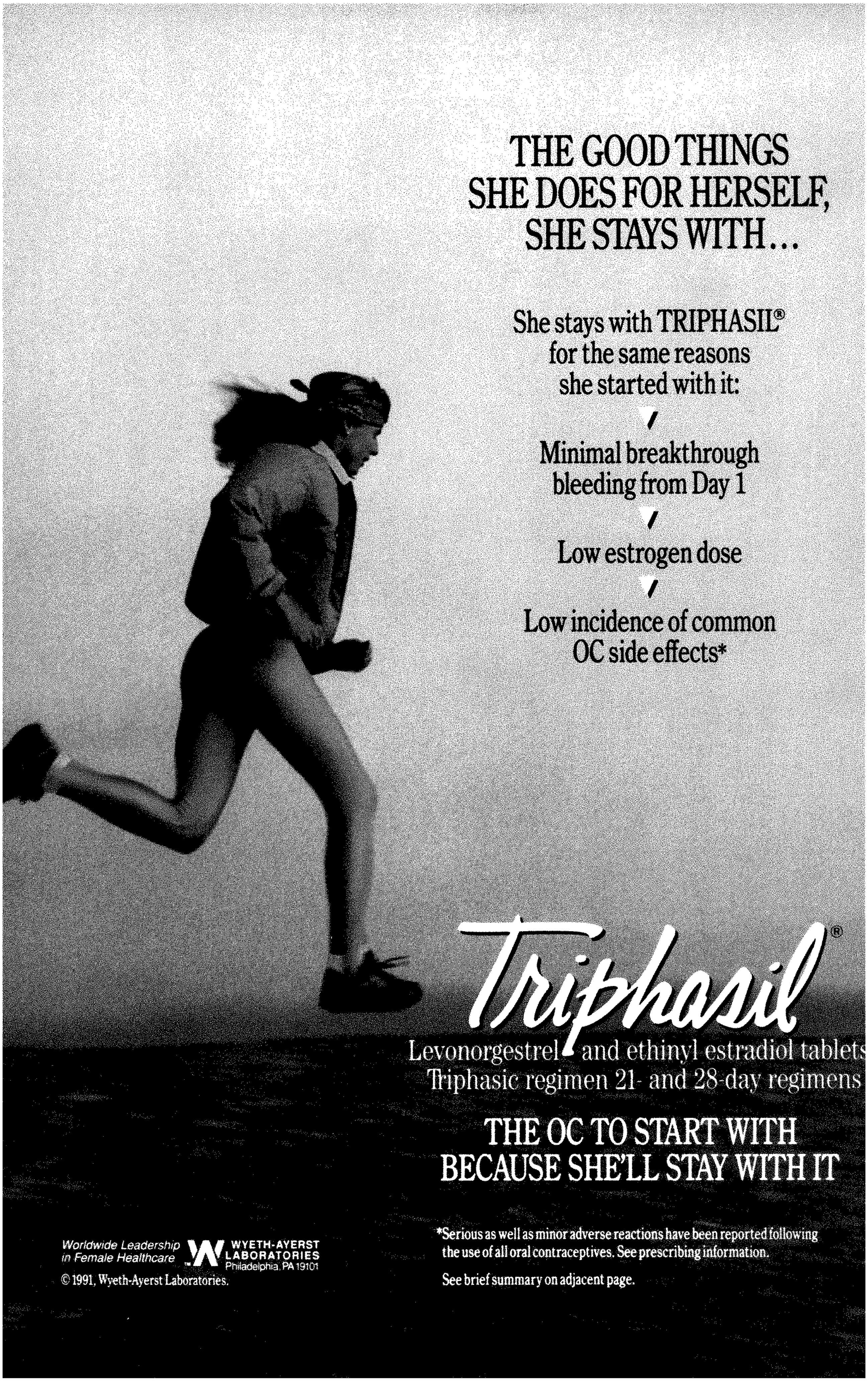

THE GOOD THINGS SHE DOES FOR HERSELF, SHE STAYS WITH…

She stays with TRIPHASIL®
for the same reasons
she started with it:

- Minimal breakthrough bleeding from Day 1
- Low estrogen dose
- Low incidence of common OC side effects*

Triphasil®

Levonorgestrel and ethinyl estradiol tablets
Triphasic regimen 21- and 28-day regimens

THE OC TO START WITH BECAUSE SHE'LL STAY WITH IT

*Serious as well as minor adverse reactions have been reported following the use of all oral contraceptives. See prescribing information.

See brief summary on adjacent page.

Worldwide Leadership
in Female Healthcare

WYETH-AYERST
LABORATORIES
Philadelphia, PA 19101

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Triphasil®

Levonorgestrel and ethinyl estradiol tablets—
Triphasic regimen 21- and 28-day regimens

THE OC TO START WITH BECAUSE SHE'LL STAY WITH IT

IN BRIEF:
TRIPHASIL® — 6 brown tablets containing 0.050 mg levonorgestrel with 0.030 mg ethinyl estradiol; 5 white tablets containing 0.075 mg levonorgestrel with 0.040 mg ethinyl estradiol; 10 light-yellow tablets containing 0.125 mg levonorgestrel with 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day regimen) — Triphasic regimen.

Indications and Usage — TRIPHASIL® is indicated for the prevention of pregnancy in women who elect to use oral contraceptives (OC's) as a method of contraception.

Contraindications — OC's should not be used in women with any of the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep-vein thrombophlebitis or thromboembolic disorders. 3. Cerebral-vascular or coronary-artery disease. 4. Known or suspected carcinoma of the breast. 5. Known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Known or suspected pregnancy (see Warning No. 5) 8. Benign or malignant liver tumor which developed during use of OC's or other estrogen-containing products.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.
The use of oral contraceptives is associated with increased risk of several serious conditions, including thromboembolism, stroke, myocardial infarction, hepatic adenoma, gallbladder disease, hypertension. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks.

1. *Thromboembolic Disorders and Other Vascular Problems* — An increased risk of thromboembolic and thrombotic disease associated with use of OC's is well established. Three principal studies in Great Britain and 3 in the U.S. have demonstrated increased risk of fatal and nonfatal venous thromboembolism and stroke, both hemorrhagic and thrombotic. These studies estimate that users of OC's are 4 to 11 times more likely than nonusers to develop these diseases without evident cause.

CEREBROVASCULAR DISORDERS — In a collaborative American study of cerebrovascular disorders in women with and without predisposing causes, it was estimated that the risk of hemorrhagic stroke was 2.0 times greater in users than nonusers and the risk of thrombotic stroke was 4 to 9.5 times greater in users than in nonusers.

MYOCARDIAL INFARCTION (MI) — An increased risk of MI associated with the use of OC's has been reported, confirming a previously suspected association. These studies, conducted in the UK, found, as expected, that the greater the number of underlying risk factors for coronary-artery disease (cigarette smoking, hypertension, hypercholesterolemia, obesity, diabetes, history of pre-eclamptic toxemia) the higher the risk of developing MI, regardless of whether the patient was an OC user or not. OC's, however, were found to be a clear additional risk factor. In terms of relative risk, it has been estimated that OC users who do not smoke (smoking is considered a major predisposing condition to MI) are about twice as likely to have a fatal MI as nonusers who do not smoke. OC users who are also smokers have about a 5-fold increased risk of fatal MI compared to users who do not smoke, but about a 10- to 12-fold increased risk compared to nonusers who do not smoke. Furthermore, amount of smoking is also an important factor. In determining importance of these relative risks, however, baseline rates for various age groups must be given serious consideration. Importance of other predisposing conditions mentioned above in determining relative and absolute risks has not as yet been quantified; quite likely the same synergistic action exists, but perhaps to a lesser extent.

RISK OF DOSE — In an analysis of data derived from several national adverse-reaction reporting systems, British investigators concluded that risk of thromboembolism, including coronary thrombosis, is directly related to dose of estrogen in OC's. Preparations containing 100 mcg or more of estrogen were associated with higher risk of thromboembolism than those containing 50-80 mcg. Their analysis did suggest, however, that quantity of estrogen may not be the sole factor involved. This finding has been confirmed in the U.S.

ESTIMATE OF EXCESS MORTALITY FROM CIRCULATORY DISEASES — A large prospective study carried out in the UK estimated the mortality rate per 100,000 women per year from diseases of the circulatory system for users and nonusers of OC's according to age, smoking habits, and duration of use. Overall excess death rate annually from circulatory diseases for OC users was estimated to be 20 per 100,000 (ages 15-34 — 5/100,000; ages 35-44 — 33/100,000; ages 45-49 — 140/100,000), risk being concentrated in older women, in those with long duration of use and in cigarette smokers. It was not possible, however, to examine interrelationships of age, smoking, and duration of use, nor to compare effects of continuous vs. intermittent use. Although the study showed a 10-fold increase in death due to circulatory diseases in users for 5 or more years, all these deaths occurred in women 35 or older. Until larger numbers of women under 35 with continuous use for 5 or more years are available, it is not possible to assess magnitude of relative risk for this younger group. Available data from a variety of sources have been analyzed to estimate risk of death associated with various methods of contraception. Estimates of risk of death for each method include combined risk of contraceptive method (e.g., thromboembolic and thrombotic disease in the case of OC's) plus risk attributable to pregnancy or abortion in event of method failure. This latter risk varies with effectiveness of method. The study concluded that mortality associated with all methods of birth control is low and below that associated with childbirth, with the exception of OC's in women over 40 who smoke. Lowest mortality is associated with condom or diaphragm backed up by early abortion. Risk of thromboembolic and thrombotic disease associated with OC's increases with age after about 30 and, for MI, is further increased by hypertension, hypercholesterolemia, obesity, diabetes, or history of pre-eclamptic toxemia, and especially cigarette smoking. Physician and patient should be alert to earliest manifestations of thromboembolic and thrombotic disorders (e.g., thrombophlebitis, pulmonary embolism, cerebrovascular insufficiency, coronary occlusion, retinal thrombosis, and mesenteric thrombosis). Should any of these occur or be suspected, the drug should be discontinued immediately. A 4- to 6-fold increased risk of postsurgery thromboembolic complications has been reported in OC users. If feasible, OC's should be discontinued at least 4 weeks before surgery of a type associated with increased risk of thromboembolism or prolonged immobilization.

PERSISTENCE OF RISK OF VASCULAR DISORDERS — Findings from one study in Britain involving cerebrovascular disease and another in the U.S. concerning MI suggest an increased risk of these conditions in users of OC's persists after discontinuation of the OC's. In the British study, risk of cerebrovascular disease remained elevated in former OC users for at least 6 years after discontinuation. In the U.S. study, increased risk of MI persisted for at least 9 years in women 40 to 49 years old who had used OC's for 5 or more years. Findings in both studies require confirmation since they are inconsistent with other published information.

2. *Ocular Lesions* — There have been reports of neuro-ocular lesions such as optic neuritis or retinal thrombosis associated with use of OC's. Discontinue OC's if there is unexplained, sudden or gradual, partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal-vascular lesions, and institute appropriate diagnostic and therapeutic measures.

3. *Carcinoma* — Long-term continuous administration of either natural or synthetic estrogen in certain animal species increases frequency of carcinoma of the breast, cervix, vagina, and liver. Certain synthetic progestogens, none currently contained in OC's, have been noted to increase incidence of mammary nodules, benign and malignant, in dogs. In humans, 3 case-control studies have reported an increased risk of endometrial carcinoma associated with prolonged use of exogenous estrogen in postmenopausal women. One publication reported on the first 21 cases submitted by physicians to a registry of cases of adenocarcinoma of the endometrium in women under 40 on OC's. Of cases found in women without predisposing risk factors (e.g., irregular bleeding at the time OC's were first given, polycystic ovaries), nearly all occurred in women who had used a sequential OC. These are no longer marketed. No evidence has been reported suggesting increased risk of endometrial cancer in users of conventional combination or progestogen-only OC's. Several studies have found no increase in breast cancer in women taking OC's or estrogens. One study, however, while also noting no overall increased risk of breast cancer in women on OC's, found an excess risk in subgroups of OC users with documented benign breast disease. Reduced occurrence of benign breast tumors in users of OC's has been well documented. In summary, there is at present no confirmed evidence from human studies of increased risk of cancer associated with OC's. Close clinical surveillance of all women on OC's is, nevertheless, essential. In all cases of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be taken to rule out malignancy. Women with a strong family history of breast cancer or with breast nodules, fibrocystic disease, or abnormal mammograms should be monitored with particular care if they elect to use OC's.

4. *Hepatic Tumors* — Benign hepatic adenomas have been found to be associated with use of OC's. One study showed that OC's with high hormonal potency were associated with higher risk than lower potency OC's. Although benign, hepatic adenomas may rupture and may cause death through intra-abdominal hemorrhage. This has been reported in short-term as well as long-term users. Two studies relate risk with duration of use of OC's, the risk being much greater after 4 or more years' use. While hepatic adenoma is rare, it should be considered in women presenting abdominal pain and tenderness, abdominal mass or shock. A few cases of hepatocellular carcinoma have been reported in women on OC's. Relationship of these drugs to this type of malignancy is not known.

5. *Use in or Immediately Preceding Pregnancy, Birth Defects in Offspring, and Malignancy in Female Offspring* — Use of female sex hormones — both estrogenic and progestational agents — during early pregnancy may seriously damage the offspring. It has been shown that females exposed in utero to diethylstilbestrol, a nonsteroidal estrogen, have increased risk of developing in later life a form of vaginal or cervical cancer ordinarily extremely rare. This risk has been estimated to be of the order of 1 in 1,000 exposures or less. Although there is no evidence now that OC's further enhance risk of developing this type of malignancy, such patients should be monitored with particular care if they elect to use OC's. Furthermore, 30 to 90% of such exposed women have been found to have epithelial changes c
vagina and cervix. Although these changes are histologically benign, it is not known whether this condition i
precursor of vaginal malignancy. Male children so exposed may develop abnormalities of the urogenital tract
similar data are not available with use of other estrogens, it cannot be presumed they would not induce simi
changes. An increased risk of congenital anomalies, including heart defects and limb defects, has been rep
use of sex hormones, including OC's, in pregnancy. One case-control study estimated a 4.7-fold increase in
limb-reduction defects in infants exposed in utero to sex hormones (OC's, hormonal withdrawal tests for pre
attempted treatment for threatened abortion). Some exposures involved only a few days. Data suggest that
limb-reduction defects in exposed fetuses is somewhat less than 1 in 1,000 live births. In the past, female
hormones have been used during pregnancy in an attempt to treat threatened or habitual abortion. There is
considerable evidence that estrogens are ineffective for these indications, and there is no evidence from we
controlled studies that progestogens are effective. There is some evidence that triploidy and possibly other
polyploidy are increased among abortuses from women who become pregnant soon after ceasing OC's. Em
these anomalies are virtually always aborted spontaneously. Whether there is an overall increase in spontan
abortion of pregnancies conceived soon after stopping OC's is unknown. It is recommended that, for any pa
has missed 2 consecutive periods, pregnancy should be ruled out before continuing OC's. If the patient has
adhered to the prescribed schedule, the possibility of pregnancy should be considered at time of first misse
and further use of OC's should be withheld until pregnancy has been ruled out. If pregnancy is confirmed, th
should be apprised of the potential risks to the fetus, and advisability of continuation of the pregnancy shou
discussed. It is also recommended that women who discontinue OC's with intent of becoming pregnant use
alternate form of contraception for a period of time before attempting to conceive. Many clinicians recomme
months, although no precise information is available on which to base this. The administration of progesto
combinations to induce withdrawal bleeding should not be used as a test of pregnancy.

6. *Gallbladder Disease* — Studies report increased risk of surgically confirmed gallbladder disease in users
estrogens. In one study, increased risk appeared after 2 years' use and doubled after 4 or 5 years' use. In o
other studies, increased risk was apparent between 6 and 12 months' use.

7. *Carbohydrate and Lipid Metabolic Effects* — Decrease in glucose tolerance has been observed in a signifi
percentage of patients on OC's. For this reason, prediabetic and diabetic patients should be carefully obser
OC's. Increases in triglycerides and total phospholipids have been observed in patients on OC's. Three stud
performed with Triphasil and no significant alterations in lipid metabolism were noted except for a slight inc
triglyceride levels in 1 study. Clinical significance of these findings remains to be defined.

8. *Elevated Blood Pressure* — Increase in blood pressure has been reported in patients on OC's. In some wo
hypertension may occur within a few months of beginning OC's. In the 1st year of use, prevalence of women
hypertension is low in users and may be no higher than that of a comparable group of nonusers. Prevalence
increases, however, with longer exposure, and in the 5th year of use is 2½ to 3 times the reported prevalen
1st year. Age is also strongly correlated with development of hypertension in OC users. Women who previou
had hypertension during pregnancy may be more likely to develop elevation of blood pressure on OC's. Hype
that develops as a result of taking OC's usually returns to normal after discontinuing the drug.

9. *Headache* — Onset or exacerbation of migraine or development of headache of a new pattern which is rec
persistent, or severe, requires discontinuation of OC's and evaluation of the cause.

10. *Bleeding Irregularities* — Breakthrough bleeding, spotting, and amenorrhea are frequent reasons for patie
discontinuing OC's. In breakthrough bleeding, as in all cases of irregular vaginal bleeding, nonfunctional cau
be borne in mind. In undiagnosed persistent or recurrent abnormal bleeding from the vagina, adequate diagn
measures are indicated to rule out pregnancy or malignancy. If pathology has been excluded, time or change
OC may solve the problem. Changing to an OC with a higher estrogen content, while potentially useful in mi
menstrual irregularity, should be done only if necessary, since this may increase risk of thromboembolic dise
Women with past history of oligomenorrhea or secondary amenorrhea or young women without regular cycle
a tendency to remain anovulatory or to become amenorrheic after discontinuing OC's. Women with these pre
problems should be advised of this possibility and encouraged to use other methods. Post-use anovulation,
prolonged, may also occur in women without previous irregularities.

11. *Ectopic Pregnancy* — Ectopic as well as intrauterine pregnancy may occur in contraceptive failures.

12. *Breast-feeding* — OC's given in the postpartum period may interfere with lactation and decrease quantity
of breast milk. Furthermore, a small fraction of the hormones in OC's has been identified in the milk of moth
OC's; effects, if any, on the breast-fed child have not been determined. If feasible, defer OC's until infant has b

Precautions — GENERAL — 1. A complete medical and family history should be taken prior to initiation of
Pretreatment and periodic physical examinations should include special reference to blood pressure, breast
and pelvic organs, including Pap smear and relevant laboratory tests. As a general rule OC's should not be p
for longer than 1 year without another physical examination and Pap smear. 2. Under influence of estrogen-p
preparations, preexisting uterine leiomyomata may increase in size. 3. Patients with history of psychic depre
should be carefully observed and the drug discontinued if depression recurs to a serious degree. Patients be
significantly depressed while on OC's should stop OC's and use an alternate method to try to determine wh
symptom is drug-related. 4. OC's may cause some degree of fluid retention. They should be prescribed with
and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention, su
convulsive disorders, migraine syndrome, asthma, or cardiac or renal insufficiency. 5. Patients with a past hi
jaundice during pregnancy have an increased risk of recurrence while on OC's. If jaundice develops, OC's s
discontinued. 6. Steroid hormones may be poorly metabolized in patients with impaired liver function and sh
administered with caution. 7. OC users may have disturbances in normal tryptophan metabolism which may
relative pyridoxine deficiency. Clinical significance is undetermined. 8. Serum folate levels may be depresse
OC's. Since the pregnant woman is predisposed to development of folate deficiency and incidence of folate
increases with increasing gestation, it is possible that if a woman becomes pregnant shortly after stopping
may have a greater chance of developing folate deficiency and complications attributed to this deficiency. 9.
pathologist should be advised of OC therapy when relevant specimens are submitted. 10. Certain endocrine
liver-function tests and blood components may be affected by estrogen-containing OC's. a. Increased sulfobro
retention. b. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased nore
induced platelet aggregability. c. Increased thyroid-binding globulin (TBG) leading to increased circulating to
hormone, as measured by protein-bound iodine (PBI), T4 by column, or T4 by radioimmunoassay. Free T3 re
is decreased, reflecting the elevated TBG; free T4 concentration is unaltered. d. Decreased pregnanediol ex
e. Reduced response to metyrapone test.

Information for the Patient — See Patient Package Labeling.

Drug Interactions — Reduced efficacy and increased incidence of breakthrough bleeding have been assoc
concomitant use of rifampin. A similar association has been suggested with barbiturates, phenylbutazone, p
sodium, ampicillin and tetracycline.

Carcinogenesis — See Warnings section for information on carcinogenesis.

Pregnancy — Category X. See Contraindications, Warnings.

Nursing Mothers — See Warnings.

Adverse Reactions — An increased risk of these serious adverse reactions has been associated with use
(see Warnings): thrombophlebitis, pulmonary embolism, coronary thrombosis, cerebral thrombosis, cerebral
hypertension, gallbladder disease, benign hepatomas, congenital anomalies. There is evidence of an associ
between the following conditions and use of OC's although additional confirmatory studies are needed: mes
thrombosis, neuro-ocular lesions, e.g., retinal thrombosis and optic neuritis.

The following adverse reactions have been reported in patients on OC's and are believed to be drug-related.
and/or vomiting, usually the most common adverse reactions, occur in approximately 10 percent or less of
during the first cycle. Other reactions, as a general rule, are seen much less frequently or only occasionally.
Gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding, spotting, chan
menstrual flow: dysmenorrhea, amenorrhea during and after treatment, temporary infertility after discontinua
treatment; edema; chloasma or melasma which may persist; breast changes: tenderness, enlargement, and
change in weight (increase or decrease); change in cervical erosion and cervical secretion, possible diminut
lactation when given immediately postpartum; cholestatic jaundice; migraine; increase in size of uterine leio
rash (allergic); mental depression; reduced tolerance to carbohydrates; vaginal candidiasis; change in corne
(steepening); intolerance to contact lenses. The following adverse reactions have been reported in users of
the association has been neither confirmed nor refuted: premenstrual-like syndrome, cataracts, changes in
chorea, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of sc
erythema multiforme, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, hemolytic uremic syndr

Acute Overdose — Serious ill effects have not been reported following acute ingestion of large doses of O
young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Dosage and Administration — For maximum contraceptive effectiveness, Triphasil must be taken exactly
directed and at intervals not over 24 hours. (If Triphasil is first taken later than first day of first menstrual cy
medication or postpartum, contraceptive reliance should not be placed on it until after the first 7 consecutiv
of use. Possibility of ovulation and conception prior to initiation of medication should be considered.)
Any time patient misses 1 or 2 brown, white or light-yellow tablets, she should also use another contracept
until she has taken a tablet daily for 7 consecutive days. For full details on dosage and administration see p
information in package insert.

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San Diego, Los Angeles, and Bellflower, California, New York, New York, Tampa, Florida, Providence, Rhode Island, Columbus, Ohio, and Washington, D.C.

The "best" gestational diabetes mellitus screening approach is the universal use of a plasma glucose level 1 hour after a 50 gm oral glucose challenge; this test, performed at least once in pregnancy, uses a threshold no higher than 140 mg/dl.

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New York, New York

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Prenatal behavioral risk screening by computer in a health maintenance organization–based prenatal care clinic

Sandra C. Lapham, MD, MPH, Michael K. Kring, BSCS, and Betty Skipper, PhD
Albuquerque, New Mexico

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Short-term variation in abnormal antenatal fetal heart rate records

P. Street, MRCS, G.S. Dawes, DM, M. Moulden, and C.W.G. Redman, MB
Oxford, England

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Maturation of the uterine cervix by repeated intracervical instillation of prostaglandin E_2

Jacques M. Milliez, MD, Denis Jannet, MD, Claudine Touboul, MD, Mahfoudh El Medjadji, MD, and Bernard J. Paniel, MD
Créteil, France

Repeated intracervical instillations of 0.25 mg of prostaglandin E_2 were performed in patients with a high-risk pregnancy with no detrimental effect to the fetus.

Hypoplastic left heart syndrome: Prenatal diagnosis, clinical profile, and management

Danielle M. Blake, MD, Joshua A. Copel, MD, and Charles S. Kleinman, MD
New Haven, Connecticut

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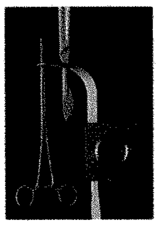

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In intra-abdominal and gynecologic infection due to indicated organisms

References: **1.** Nightingale CH, Smith KS, Quintiliani R, Briceland LL, Cooper B. The conversion of cefoxitin usage to cefotetan: an interdisciplinary approach. *Am J Surg.* 1988; 155(5A):101-102. **2.** Sochalski A, Sullman S, Andriole VT. Cost-effectiveness study of cefotetan versus cefoxitin and cefotetan versus combination antibiotic regimens. *Am J Surg.* 1988;155(5A):96-101. **3.** CEFOTAN® (cefotetan disodium) full prescribing information issued March 1986. **4.** *Physicians' Desk Reference.* ed 43. Oradell, NJ: Medical Economics Co; 1989. Mefoxin® (cefoxitin sodium, MSD). pp 1355-1357. **5.** Carver M, Quintiliani R, Nightingale CH. Comparative pharmacokinetic study of cefotetan and cefoxitin in healthy volunteers. *Infect Surg.* April 1986 (suppl), pp 11-14. **6.** Quintiliani R, Nightingale CH, Stevens RC, Outman WR, Deckers PJ, Martens MG. Comparative pharmacokinetics of cefotetan and cefoxitin in patients undergoing hysterectomies and colorectal operations. *Am J Surg.* 1988;155(5A):67-70. **7.** Centers for Disease Control. 1989 Sexually Transmitted Diseases Treatment Guidelines. *MMWR.* 1989;38(suppl S-8):1-43.

Please see adjacent page for brief summary of prescribing information.

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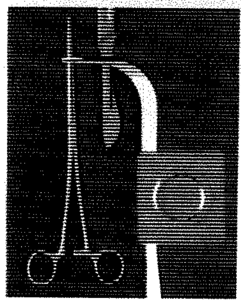

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In intra-abdominal and gynecologic infection due to indicated organisms

For Intravenous or Intramuscular Use
(FOR FULL PRESCRIBING INFORMATION, SEE PACKAGE INSERT.)

INDICATIONS AND USAGE

Treatment: CEFOTAN is indicated for the therapeutic treatment of the following infections when caused by susceptible strains of the designated organisms:

Urinary Tract Infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Proteus mirabilis*, and *Proteus* sp (which may include the organisms now called *Proteus vulgaris*, *Providencia rettgeri*, and *Morganella morganii*).

Lower Respiratory Tract Infections caused by *Streptococcus pneumoniae* (formerly *D pneumoniae*), *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Haemophilus influenzae* (including ampicillin-resistant strains), *Klebsiella* species (including *K pneumoniae*), *E coli*, *Proteus mirabilis*, and *Serratia marcescens*.*

Skin and Skin Structure Infections caused by *Staphylococcus aureus* (penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus* species (excluding enterococci), *E coli*, *Klebsiella pneumoniae*, and *Peptococcus** and *Peptostreptococcus* sp.*

Gynecologic Infections caused by *Staphylococcus aureus* (including penicillinase- and nonpenicillinase-producing strains), *Staphylococcus epidermidis*, *Streptococcus* species (excluding enterococci), group B streptococci, *E coli*, *Proteus mirabilis*, *Neisseria gonorrhoeae*, *Bacteroides* species (excluding *B distasonis*, *B ovatus*, *and B thetaiotaomicron*), *Fusobacterium* species,* and gram-positive anaerobic cocci (including *Peptococcus* and *Peptostreptococcus* species).

Intra-abdominal Infections caused by *E coli*, *Klebsiella* species (including *K pneumoniae*), *Streptococcus* species (excluding enterococci), *Bacteroides* species (excluding *B distasonis*, *B ovatus*, and *B thetaiotaomicron*), and *Clostridium* species.*

Bone and Joint Infections caused by *Staphylococcus aureus*.*

*Efficacy for this organism in this organ system was studied in fewer than ten infections.

Specimens for bacteriological examination should be obtained in order to isolate and identify causative organisms and to determine their susceptibilities to cefotetan. Therapy may be instituted before results of susceptibility studies are known; however, once these results become available, the antibiotic treatment should be adjusted accordingly.

In cases of confirmed or suspected gram-positive or gram-negative sepsis or in patients with other serious infections in which the causative organism has not been identified, it is possible to use CEFOTAN concomitantly with an aminoglycoside. Cefotetan combinations with aminoglycosides have been shown to be synergistic in vitro against many Enterobacteriaceae and also some other gram-negative bacteria. The dosage recommended in the labeling of both antibiotics may be given and depends on the severity of the infection and the patient's condition.

Note: If CEFOTAN and an aminoglycoside are used concomitantly, renal function should be carefully monitored, especially if higher dosages of the aminoglycoside are to be administered or if therapy is prolonged, because of the potential nephrotoxicity and ototoxicity of aminoglycosidic antibiotics. Although, to date, nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Prophylaxis: The preoperative administration of CEFOTAN may reduce the incidence of certain postoperative infections in patients undergoing surgical procedures that are classified as clean contaminated or potentially contaminated (eg, cesarean section, abdominal or vaginal hysterectomy, transurethral surgery, biliary tract surgery, and gastrointestinal surgery).

The prophylactic dose of CEFOTAN should be administered 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, CEFOTAN should be administered intravenously after the clamping of the umbilical cord.

If there are signs and symptoms of infection, specimens for culture should be obtained for identification of the causative organism so that appropriate therapeutic measures may be initiated.

CONTRAINDICATIONS

CEFOTAN is contraindicated in patients with known allergy to the cephalosporin group of antibiotics.

WARNINGS

Before therapy with CEFOTAN is instituted, careful inquiry should be made to determine whether the patient has had previous hypersensitivity reactions to cefotetan disodium, cephalosporins, penicillins, or other drugs. This product should be given cautiously to penicillin-sensitive patients. Antibiotics should be administered with caution to any patient who has demonstrated some form of allergy, particularly to drugs. If an allergic reaction to CEFOTAN occurs, discontinue the drug. Serious acute hypersensitivity reactions may require epinephrine and other emergency measures.

Pseudomembranous colitis has been reported with the use of cephalosporins (and other broad-spectrum antibiotics); therefore, it is important to consider its diagnosis in patients who develop diarrhea in association with antibiotic use.

Treatment with broad-spectrum antibiotics may alter normal flora of the colon and may permit overgrowth of clostridia. Studies indicate a toxin produced by *Clostridium difficile* is one primary cause of antibiotic-associated colitis.

Mild cases of colitis may respond to drug discontinuance alone. Moderate to severe cases should be managed with fluid, electrolyte, and protein supplementation as indicated. When the colitis is not relieved by drug discontinuance, or when it is severe, oral vancomycin is the treatment of choice for antibiotic-associated pseudomembranous colitis produced by *C difficile*. Other causes should also be considered.

In common with many other broad-spectrum antibiotics, CEFOTAN may be associated with a fall in prothrombin activity and, possibly, subsequent bleeding. Those at increased risk include patients with renal or hepatobiliary impairment or poor nutritional state, the elderly, and patients with cancer. Prothrombin time should be monitored and exogenous vitamin K administered as indicated.

PRECAUTIONS

General: As with other broad-spectrum antibiotics, prolonged use of CEFOTAN may result in overgrowth of nonsusceptible organisms. Careful observation of the patient is essential. If superinfection does occur during therapy, appropriate measures should be taken.

CEFOTAN should be used with caution in individuals with a history of gastrointestinal disease, particularly colitis.

Information for Patients: As with some other cephalosporins, a disulfiram-like reaction characterized by flushing, sweating, headache, and tachycardia may occur when alcohol (beer, wine, etc.) is ingested within 72 hours after CEFOTAN administration. Patients should be cautioned about the ingestion of alcoholic beverages following the administration of CEFOTAN.

Drug Interactions: Although to date nephrotoxicity has not been noted when CEFOTAN was given alone, it is possible that nephrotoxicity may be potentiated if CEFOTAN is used concomitantly with an aminoglycoside.

Drug/Laboratory Test Interactions: A false positive reaction for glucose in urine may occur with Benedict's or Fehling's solution.

As with other cephalosporins, high concentrations of cefotetan may interfere with measurement of serum and urine creatinine levels by Jaffe reaction and produce false increases in the levels of creatinine reported.

Carcinogenesis, Mutagenesis, Impairment of Fertility: Although long-term studies in animals have not been performed to evaluate carcinogenic potential, no mutagenic potential of cefotetan was found in standard laboratory tests.

Cefotetan has adverse effects on the testes of prepubertal rats. Subcutaneous administration of 500 mg/kg/day (approximately 8-16 times the usual adult human dose) on days 6-35 of life (thought to be developmentally analogous to late childhood and prepuberty in humans) resulted in reduced testicular weight and seminiferous tubule degeneration in 10 of 10 animals. Affected cells included spermatogonia and spermatocytes; Sertoli and Leydig cells were unaffected. Incidence and severity of lesions were dose-dependent; at 120 mg/kg/day (approximately 2-4 times the usual human dose) only 1 of 10 treated animals was affected, and the degree of degeneration was mild.

Similar lesions have been observed in experiments of comparable design with other methylthiotetrazole-containing antibiotics and impaired fertility has been reported, particularly at high dose levels. No testicular effects were observed in 7-week-old rats treated with up to 1000 mg/kg/day SC for 5 weeks, or in infant dogs (3 weeks old) that received up to 300 mg/kg/day IV for 5 weeks. The relevance of these findings to humans is unknown.

Usage in Pregnancy: Pregnancy Category B: Reproduction studies have been performed in rats and monkeys at doses up to 20 times the human dose and have revealed no evidence of impaired fertility or harm to the fetus due to cefotetan. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproductive studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Usage in Nursing Mothers: Cefotetan is excreted in human milk in very low concentrations. Caution should be exercised when cefotetan is administered to a nursing woman.

Pediatric Use: Safety and effectiveness in children have not been established.

ADVERSE REACTIONS

In clinical studies, the following adverse effects were considered related to CEFOTAN therapy.

Gastrointestinal symptoms occurred in 1.5% of patients; the most frequent were diarrhea (1 in 80) and nausea (1 in 700).

Hematologic laboratory abnormalities occurred in 1.4% of patients and included eosinophilia (1 in 200), positive direct Coombs' test (1 in 250), and thrombocytosis (1 in 300).

Hepatic enzyme elevations occurred in 1.2% of patients and included a rise in SGPT (1 in 150), SGOT (1 in 300), alkaline phosphatase (1 in 700), and LDH (1 in 700).

Hypersensitivity reactions were reported in 1.2% of patients and included rash (1 in 150) and itching (1 in 700).

Local effects were reported in less than 1.0% of patients and included phlebitis at the site of injection (1 in 300), and discomfort (1 in 500).

During postmarketing experience with CEFOTAN, agranulocytosis, anaphylactic reactions, fever, hemolytic anemia, leukopenia, prolonged prothrombin time with or without bleeding, pseudomembranous colitis, and transient thrombocytopenia have been reported.

DOSAGE AND ADMINISTRATION

Treatment: The usual adult dosage is 1 or 2 grams of CEFOTAN administered intravenously or intramuscularly every 12 hours for 5 to 10 days. Proper dosage and route of administration should be determined by the condition of the patient, severity of the infection, and susceptibility of the causative organism.

GENERAL GUIDELINES FOR DOSAGE OF CEFOTAN

Type of Infection	Daily Dose	Frequency and Route
Urinary Tract	1-4 grams	500 mg every 12 hours IV or IM 1 or 2 g every 24 hours IV or IM 1 or 2 g every 12 hours IV or IM
Other Sites	2-4 grams	1 or 2 g every 12 hours IV or IM
Severe	4 grams	2 g every 12 hours IV
Life-Threatening	6* grams	3 g every 12 hours IV

*Maximum daily dosage should not exceed 6 grams.

Prophylaxis: To prevent postoperative infection in clean contaminated or potentially contaminated surgery in adults, the recommended dosage is 1 or 2 g of CEFOTAN administered once, intravenously, 30 to 60 minutes prior to surgery. In patients undergoing cesarean section, the dose should be administered as soon as the umbilical cord is clamped.

Impaired Renal Function: When renal function is impaired, a reduced dosage schedule must be employed. The following dosage guidelines may be used.

DOSAGE GUIDELINES FOR PATIENTS WITH IMPAIRED RENAL FUNCTION

Creatinine Clearance mL/min	Dose	Frequency
>30	Usual Recommended Dosage*	Every 12 hours
10-30	Usual Recommended Dosage*	Every 24 hours
<10	Usual Recommended Dosage*	Every 48 hours

*Dose determined by the type and severity of infection, and susceptibility of the causative organism.

Alternatively, the dosing interval may remain constant at 12 hour intervals, but the dose reduced to one-half the usual recommended dose for patients with a creatinine clearance of 10-30 mL/min, and one-quarter the usual recommended dose for patients with a creatinine clearance of less than 10 mL/min.

When only serum creatinine levels are available, creatinine clearance may be calculated from the following formula. The serum creatinine level should represent a steady state of renal function.

$$\text{Males:} \quad \frac{\text{Weight (kg)} \times (140 - \text{age})}{72 \times \text{serum creatinine (mg/100 mL)}}$$

$$\text{Females:} \quad 0.9 \times \text{value for males}$$

Cefotetan is dialyzable and it is recommended that for patients undergoing intermittent hemodialysis, one-quarter of the usual recommended dose be given every 24 hours on days between dialysis and one-half the usual recommended dose on the day of dialysis.

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Frank Gonzalez, MD, Denise A. Hatala, BS, and Leon Speroff, MD
Cleveland, Ohio

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Carl P. Weiner, MD, Roger A. Williamson, MD, Katharine D. Wenstrom, MD, Susan L. Sipes, MD, Stanley S. Grant, RN, and John A. Widness, MD
Iowa City, Iowa

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Steroid therapy for hydrops associated with antibody-mediated congenital heart block 553
William J. Watson, MD, and Vern L. Katz, MD
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Prognostic factors and complication rates for cervical cerclage: A review of 482 cases 555
Marjorie C. Treadwell, MD, Richard A. Bronsteen, MD, and Sidney F. Bottoms, MD
Detroit, Michigan

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Camran Nezhat, MD, and Farr Nezhat, MD
Atlanta, Georgia

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Use of hydroxyurea in chronic myeloid leukemia during pregnancy: A case report 565
M. Patel, MB, ChB, MMed(Wits), I.A.F. Dukes, MB, ChB, and J.C. Hull, MB, ChB
Johannesburg, South Africa

Use of hydroxyurea in chronic myeloid leukemia during pregnancy resulted in a successful outcome for both mother and fetus.

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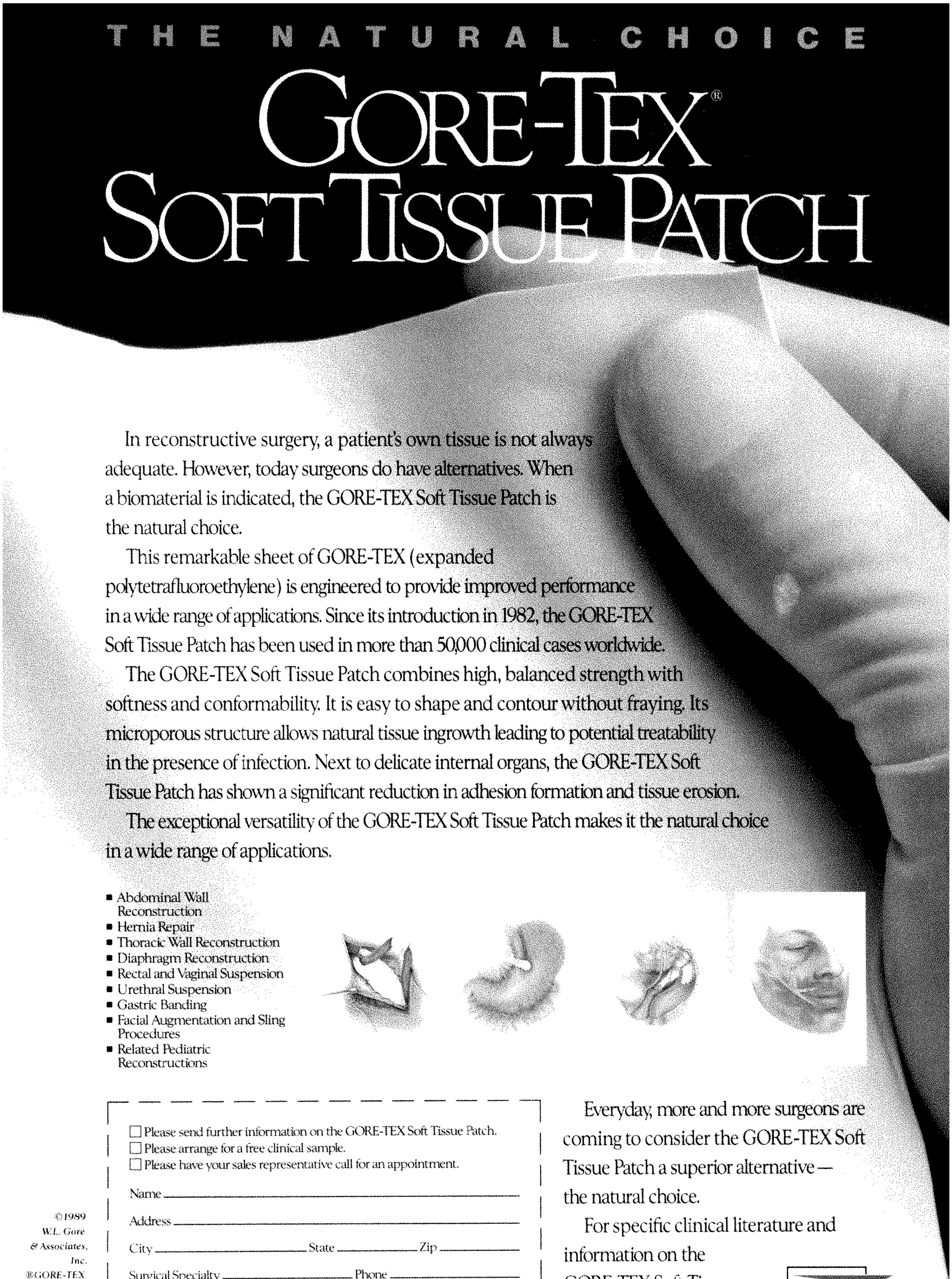

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David L. Vesely, MD, PhD

Tampa, Florida

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David H. Moore, MD, Wesley C. Fowler, Jr., MD, Connie P. Jones, BS, and Linda S. Crumpler, RN

Chapel Hill, North Carolina

Hexamethylmelamine is a well-tolerated drug with activity against ovarian cancer previously treated with cisplatin.

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Peter Schreyer, MD, Naomi Bar-Natan, MD, Dan J. Sherman, MD, Shlomo Arieli, MD, and Eliahu Caspi, MD

Zerifin, Israel

Fetal breathing movements recorded before induction of labor in postdate pregnancies are a reliable indicator of the time and quantity of oxytocin required throughout labor.

Risks associated with an elevated maternal serum α-fetoprotein level 581

Barbara F. Crandall, MD, Linda Robinson, MS, and Peggy Grau, MD

Los Angeles, California

Risks for both fetal abnormality and adverse pregnancy outcome increase directly with maternal serum α-fetoprotein levels.

Study of 156 cases of polyhydramnios and congenital malformations in a series of 118,265 consecutive births 586

Claude G. Stoll, MD, Yves Alembik, MD, and Beatrice Dott, MD

Strasbourg, France

The diagnosis of congenital malformations was performed in only 41% of 156 cases of polyhydramnios associated with congenital anomalies.

Regulation of atrial natriuretic peptide release in pregnancy: Responses to posture 591

Sandra A. Lowe, MB, Graham J. Macdonald, MD, and Mark A. Brown, MD

Sydney, Australia

Atrial natriuretic peptide is stimulated in normal pregnancy by prolonged recumbency, inhibited by upright posture, and unchanged by supine posture.

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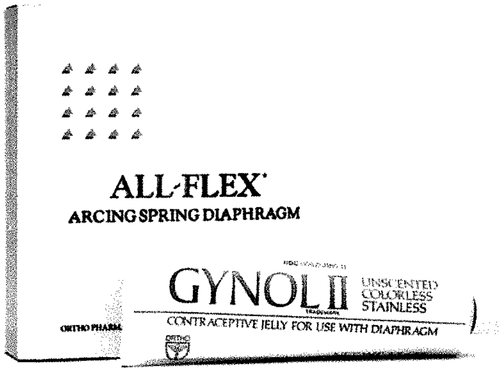
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Wilhelm Kuhnz, PhD, Durda Sostarek, Christiane Gansau, Tom Louton, PhD, and Marianne Mahler, MD
Berlin, Germany

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Jean M. Ricci, MD, Seemanthini Hariharan, MD, Andrew Helfgott, MD, Karel Reed, RN, and Mary Jo O'Sullivan, MD
Miami, Florida, and Los Angeles, California

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Dean S. Cunningham, MD, PhD, and Edward E. Evans, MD
Portsmouth, Virginia

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Duane E. Townsend, MD, Solon D. Barbis, MD, and Roger D. Mathews, MD
Sacramento, California

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G.S. Dawes, DM, S.K. Rosevear, MD, L.C. Pello, MD, M. Moulden, and C.W.G. Redman, MD
Oxford, England

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Joseph F. Polak, MD, and Diane L. Wilkinson, MD
Boston, Massachusetts

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Alexander D. Kofinas, MD, Nicolas V. Simon, MD, Henry Sagel, DO, Ellen Lyttle, RN, Nancy Smith, RN, and Karen King, RDMS
York, Pennsylvania

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James A. McGregor, MD, CM, Janice I. French, CNM, MS, and Kyung Seo, MD, PhD
Denver, Colorado

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F.L. Tyson, PhD, C.M. Boyer, PhD, R. Kaufman, MD, K. O'Briant, MS, G. Cram, BS, J.R. Crews, MD, J.T. Soper, MD, L. Daly, BHS, W.C. Fowler, Jr., MD, J.S. Haskill, PhD, and R.C. Bast, Jr., MD
Durham and Chapel Hill, North Carolina

The oncogenes c-*erb*B-2, c-*fms*, c-*myc*, n-*myc*, c-*fos*, and c-Ha-*ras* are expressed in a large fraction of ovarian cancers and deserve further evaluation as prognostic factors.

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Steven C. Presser, MD, Frank Z. Stanczyk, PhD, and Rogerio A. Lobo, MD
Los Angeles, California

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David M. Sherer, MD, Mary Lou D'Amico, RNC, BSN, David P. Warshal, MD, Robert A. Stern, MD, Harold F. Grunert, MD, and Jacques S. Abramowicz, MD
Rochester, New York

Recurrent abruptio placentae, noted immediately after electroconvulsive therapy during pregnancy, might be explained by transient acute hypertension occurring in conjunction with this treatment.

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Jeffrey M. Barrett, MD
Lakeland, Florida

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Philadelphia, Pennsylvania

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Mark H. Bogart, PhD, O.W. Jones, MD, Robin A. Felder, PhD, Robert G. Best, PhD, Linda Bradley, PhD, William Butts, PhD, Barbara Crandall, MD, Wendy MacMahon, BS, Frank H. Wians, Jr., PhD, and Paulette V. Loeh, BS

La Jolla and Los Angeles, California, Charlottesville, Virginia, Columbia, South Carolina, Chandler, Arizona, Renton, Washington, Decatur, Georgia, and Lackland, Texas

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Karin van der Mooren, MD, Leo G. Barendregt, and Juriy W. Wladimiroff, MD, PhD

Rotterdam, The Netherlands

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Tarun K. Das, PhD, Jean-Marie Moutquin, MD, and Jean-Guy Parent, MD

Quebec City, Quebec, Canada

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Kevin R. Baker, MD, Harold P. Drutz, MD, and Mary D. Barnes, RN, BA

Toronto, Ontario, Canada

A blind, randomized prospective study investigates the effectiveness of prophylactic antibiotics in preventing bacteriuria after multichannel urodynamic investigations in 124 women.

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Hisao Osada, MD, Makoto Kawata, MD, PhD, Masao Yamada, PhD, Ko Okumura, MD, PhD, and Hiroyoshi Takamizawa, MD, PhD

Chiba and Tokyo, Japan

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Lawrence A. Dolkart, MD, and Faith T. Reimers, RNC, BS, RDMS

Elmira, New York

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Marion A.L. Didden, MD, Marcel P. Hoekstra, MD, Jos H.A. Vollebergh, MD, Andries Schellekens, PhD, and Pieter A. de Jong, PhD

Eindhoven, The Netherlands

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Expression of cytokine receptors and markers of differentiation in human papillomavirus–infected cervical tissues 696

Ronald C. McGlennen, MD, Ronald S. Ostrow, PhD, Linda F. Carson, MD, Michael S. Stanley, MD, and Anthony J. Faras, PhD

Minneapolis, Minnesota

The expression of epidermal growth factor, transferrin receptors, and cytokeratin is correlated with various histologic grades of human papillomavirus–infected cervical tissues.

Hemostatic wrapping of ruptured liver in two postpartum patients 705

Andrew B. Rittenberry, Jr., MD, Colman L. Arnold, MD, and M. Mark Taslimi, MD

Chattanooga, Tennessee

Hemostatic wrapping effectively controlled bleeding of ruptured liver in two postpartum patients.

Relationship between fetal biophysical activities and umbilical cord blood gas values 707

Anthony M. Vintzileos, MD, Alfred D. Fleming, MD, William E. Scorza, MD, Edward J. Wolf, MD, James Balducci, MD, Winston A. Campbell, MD, and John F. Rodis, MD

Farmington, Connecticut

Fetuses with absence of fetal movements or fetal tone have significantly lower cord blood pH, Po_2, bicarbonate, and base excess values and higher Pco_2 levels as compared with fetuses with nonreactive nonstress test results or absence of breathing.

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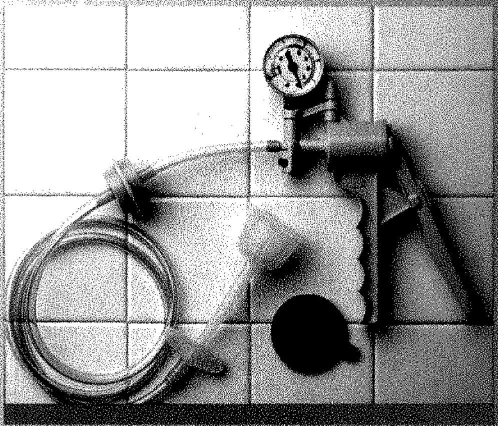

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Lauren Lynch, MD, Fernand Daffos, MD, David Emanuel, MD, Yves Giovangrandi, MD, Richard Meisel, MD, Francois Forestier, PhD, Gieri Cathomas, MD, and Richard L. Berkowitz, MD
New York, New York, and Paris, France

Prenatal diagnosis of fetal cytomegalovirus infection is possible with a combination of amniocentesis and fetal blood sampling.

Increased inducibility of inflammatory mediators from peripheral blood mononuclear cells of women with salpingitis 719

Steven S. Witkin, PhD, Miklos Toth, MD, Jan Jeremias, AB, and William J. Ledger, MD
New York, New York

Peripheral blood mononuclear cells from women with salpingitis produce monokines under conditions in which the corresponding cells from other women remain unresponsive.

Maternal plasma level of endothelin is increased in preeclampsia 724

Alfredo Nova, MD, Baha M. Sibai, MD, John R. Barton, MD, Brian M. Mercer, MD, and Murray D. Mitchell, PhD
Memphis, Tennessee, and Salt Lake City, Utah

Plasma endothelin values are increased in women with preeclampsia, particularly in those with HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count).

In utero fetal muscle biopsy for the diagnosis of Duchenne muscular dystrophy 728

Mark I. Evans, MD, Anne Greb, MS, Louis M. Kunkel, PhD, Alan J. Sacks, MD, Mark P. Johnson, MD, Corinne Boehm, MS, Haig H. Kazazian, Jr., MD, and Eric P. Hoffman, PhD
Detroit, Michigan, Boston, Massachusetts, and Baltimore, Maryland

An in utero fetal muscle biopsy specimen was obtained from a male fetus at risk for Duchenne muscular dystrophy; it was analyzed for the dystrophin protein.

Concentration of digoxin-like immunoreactive substance in patients with preeclampsia and its relation to severity of pregnancy-induced hypertension 733

Krzysztof Kaminski, MD, PhD, and Tomasz Rechberger, MD, PhD
Lublin, Poland

A positive significant correlation between maternal serum concentration of digoxin-like immunoreactive substance and severity of preeclampsia was found.

A new proline aminopeptidase assay for diagnosis of bacterial vaginosis 737

Judith N. Schoonmaker, PhD, Brenda D. Lunt, MS, David W. Lawellin, PhD, Janice I. French, CNM, MS, Sharon L. Hillier, PhD, and James A. McGregor, MDCM
Denver, Colorado, and Seattle, Washington

An improved laboratory test for proline aminopeptidase is presented for use in the diagnosis of bacterial vaginosis.

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Igal Wolman, MD, Joseph Ophir, MD, David Pauzner, MD, Sarah Brenner, MD, and Menachem P. David, MD

Tel Aviv, Israel

Pemphigus gestationis is a rare disease with a high rate of morbidity and mortality to the fetus; a report of successful treatment of this disease is presented.

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Pelvic denervation for chronic pain associated with endometriosis: Fact or fancy? 745

Paolo Vercellini, MD, Luigi Fedele, MD, Stefano Bianchi, MD, and Giovanni Battista Candiani, MD

Milan, Italy

Analysis of literature data fails to confirm the effectiveness of presacral neurectomy and amputation of uterosacral ligaments in women with chronic pelvic pain associated with endometriosis.

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Inhibin subunits in human placenta: Localization and messenger ribonucleic acid levels during pregnancy 750

Felice Petraglia, MD, Gian Carlo Garuti, MD, Laura Calza', MD, Veronica Roberts, PhD, Luciana Giardino, MD, Andrea R. Genazzani, MD, Wylie Vale, PhD, and Helene Meunier, PhD

Modena and Cagliari, Italy, La Jolla, California, and Toronto, Ontario, Canada

Human cytotrophoblast and syncytiotrophoblast cells begin to produce inhibin and activin early in gestation, with the highest production rate at term.

Circadian rhythms of heart rate and mean arterial pressure in chronically instrumented pregnant rats 758

Paula J. Melone, DO, Paul J. Meis, MD, and David A. Blizard, PhD

Winston-Salem, North Carolina

Normotensive pregnant rats monitored continuously over the last half of gestation showed statistically significant circadian rhythms in heart rate and blood pressure; blood pressure decreased near term, but heart rate increased.

Bilateral destruction of the fetal paraventricular nuclei prolongs gestation in sheep 764

Thomas J. McDonald, PhD, and Peter W. Nathanielsz, MD, PhD

Ithaca, New York

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New Haven and Groton, Connecticut

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Philadelphia, Pennsylvania

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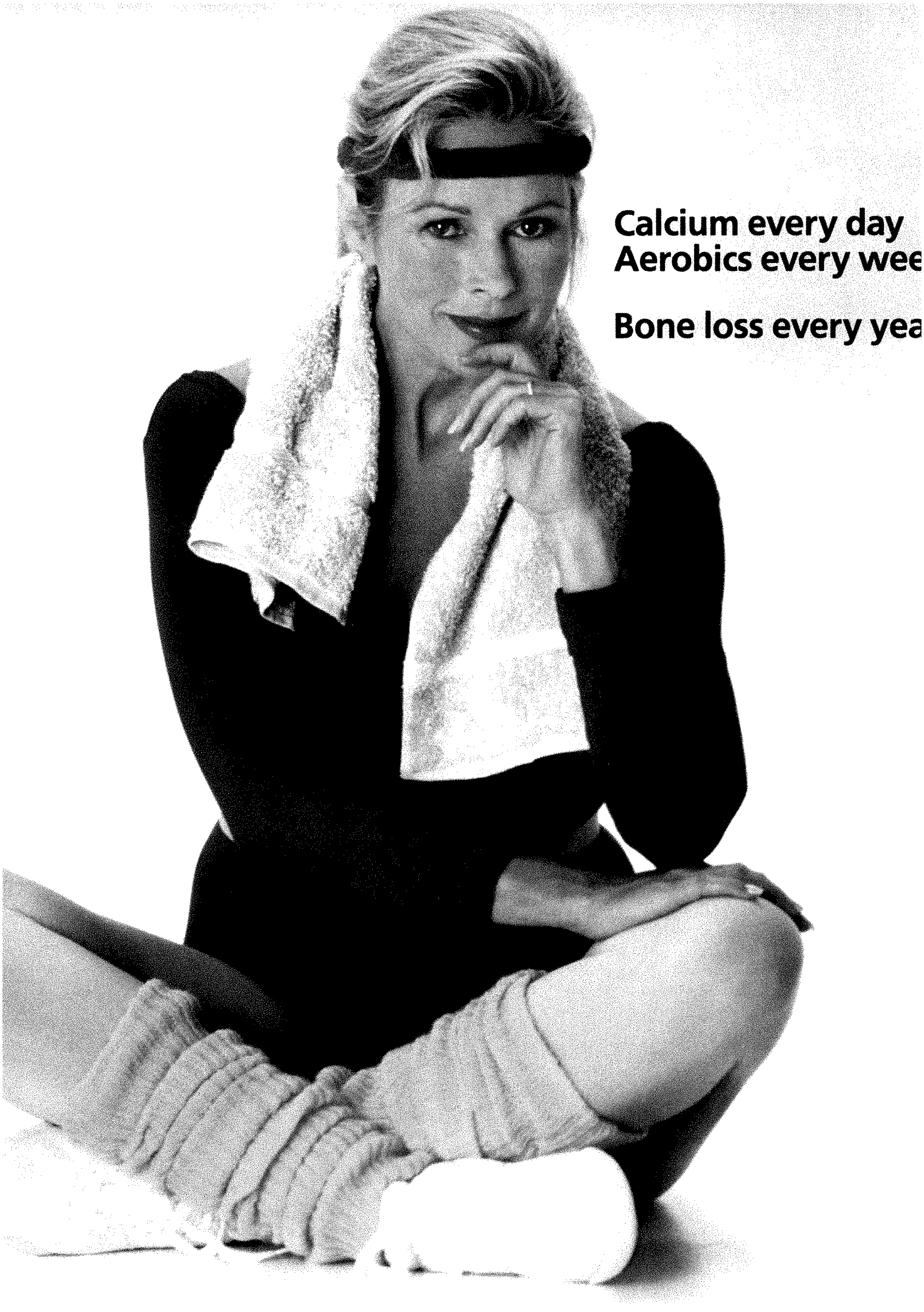
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See important information on adjacent page.

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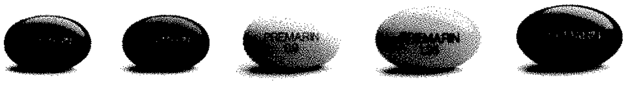

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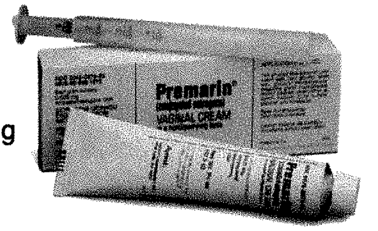

BRIEF SUMMARY *(FOR FULL PRESCRIBING INFORMATION AND PATIENT INFORMATION, SEE PACKAGE CIRCULARS.)*

PREMARIN® Brand of conjugated estrogens tablets, USP
PREMARIN® Brand of conjugated estrogens Vaginal Cream, in a nonliquefying base

1. ESTROGENS HAVE BEEN REPORTED TO INCREASE THE RISK OF ENDOMETRIAL CARCINOMA.
Close clinical surveillance of all women taking estrogens is important. Adequate diagnostic measures including endometrial sampling when indicated should be undertaken to rule out malignancy in all cases of undiagnosed persistent or recurring abnormal vaginal bleeding. There is currently no evidence that "natural" estrogens are more or less hazardous than "synthetic" estrogens at equiestrogenic doses.
2. ESTROGENS SHOULD NOT BE USED DURING PREGNANCY.
Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in the reproductive organs of the male and female fetus, an increased risk of vaginal adenosis, squamous-cell dysplasia of the uterine cervix, and vaginal cancer in the female later in life. The 1985 DES Task Force concluded that women who used DES during their pregnancies may subsequently experience an increased risk of breast cancer. However, a causal relationship is still unproven, and the observed level of risk is similar to that for a number of other breast cancer risk factors.

There is no indication for estrogen therapy during pregnancy. Estrogens are ineffective for the prevention or treatment of threatened or habitual abortion.

DESCRIPTION: PREMARIN (conjugated estrogens, USP) contains a mixture of estrogens, obtained exclusively from natural sources, blended to represent the average composition of material derived from pregnant mares' urine. It contains estrone, equilin, and 17α-dihydroequilin, together with smaller amounts of 17α-estradiol, equilenin, and 17α-dihydroequilenin as salts of their sulfate esters. Tablets are available in 0.3 mg, 0.625 mg, 0.9 mg, 1.25 mg, and 2.5 mg strengths of conjugated estrogens. Cream is available as 0.625 mg conjugated estrogens per gram.

INDICATIONS AND USAGE: Moderate-to-severe vasomotor symptoms associated with the menopause. (There is no evidence that estrogens are effective for nervous symptoms or depression which might occur during menopause and they should not be used to treat these conditions.) Prevention and management of osteoporosis (abnormally low bone mass). Atrophic vaginitis. Atrophic urethritis. Hypoestrogenism due to hypogonadism, castration or primary ovarian failure.

PREMARIN (conjugated estrogens) Vaginal Cream is indicated in the treatment of atrophic vaginitis and kraurosis vulvae.

PREMARIN HAS NOT BEEN SHOWN TO BE EFFECTIVE FOR ANY PURPOSE DURING PREGNANCY AND ITS USE MAY CAUSE SEVERE HARM TO THE FETUS (SEE BOXED WARNING).

CONTRAINDICATIONS: Estrogens should not be used in women (or men) with any of the following conditions: 1. Known or suspected pregnancy (see Boxed Warning). 2. Known or suspected cancer of the breast except in appropriately selected patients being treated for metastatic disease. 3. Known or suspected estrogen-dependent neoplasia. 4. Undiagnosed abnormal genital bleeding. 5. Active thrombophlebitis or thromboembolic disorders. 6. Estrogen replacement therapy has not been reported to increase the risk of thrombophlebitis and/or thromboembolic disease. However, there is insufficient information regarding women who have had previous thromboembolic disease.

PREMARIN Tablets and Vaginal Cream should not be used in patients hypersensitive to their ingredients.

WARNINGS: Some studies suggest a possible increased incidence of breast cancer in women taking higher doses of estrogen for prolonged time periods. The majority of studies have not shown an association with usual estrogen replacement doses. Endometrial cancer risk among estrogen users was about 4-fold or greater than in non-users, and appears dependent on treatment duration and estrogen dose. In patients on combined estrogen-progestin therapy, this risk appears to be decreased. (See PRECAUTIONS below.)

Estrogen therapy during pregnancy is associated with an increased risk of fetal congenital reproductive tract disorders.

A 2.5-fold increase in the risk of surgically confirmed gall bladder disease in women receiving postmenopausal estrogens has been reported.

Large doses of estrogen such as those used to treat prostate and breast cancer have been shown to increase the risk of non-fatal myocardial infarction, pulmonary embolism, and thrombophlebitis in men. This cannot necessarily be extrapolated to women. However, to avoid theoretical cardiovascular risk caused by high estrogen doses, the doses for estrogen replacement therapy should not exceed the recommended dose.

Blood pressure should be monitored with estrogen use, especially if high doses are used.

Estrogens may lead to severe hypercalcemia in patients with breast cancer and bone metastases.

PRECAUTIONS: The addition of a progestin for 7 or more days of a cycle of estrogen administration reportedly lowers the incidence of endometrial hyperplasia. Studies of endometrium suggest that 10 to 13 days of progestin are needed to provide maximal endometrial maturation and elimination of hyperplastic changes. Additional risk, such as adverse effects on carbohydrate and lipid metabolism, may be associated with the inclusion of progestin in estrogen replacement regimens. The choice of progestin and dosage may be important in minimizing these adverse effects.

Physical examination and a complete medical and family history should be taken prior to the initiation of any estrogen therapy with special reference to blood pressure, breasts, abdomen, and pelvic organs, and should include a Papanicolaou smear. As a general rule, estrogen should not be prescribed for longer than one yea without another physical examination being performed. Conditions influenced by fluid retention, such as asthma, epilepsy, migraine, and cardiac or renal dysfunction, require careful observation. Certain patients may develo manifestations of excessive estrogenic stimulation, such as abnormal or excessive uterine bleeding an mastodynia. Pre-existing uterine leiomyomata may increase in size during estrogen use. Estrogens should b used with care in patients with impaired liver function, renal insufficiency, or metabolic bone diseases associate with hypercalcemia.

The following drug/laboratory test interactions have been reported, some only with estrogen-progesti combinations (oral contraceptives):

1. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3; increased norepinephrine-induced platelet aggregability.
2. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, a measured by T_4 levels determined by column or by radioimmunoassay. Free T_3 resin uptake is decreased reflecting the elevated TBG; free T_4 concentration is unaltered.
3. Impaired glucose tolerance.
4. Reduced response to metyrapone test.
5. Reduced serum folate concentration.

MUTAGENESIS AND CARCINOGENESIS: Long-term, continuous administration of natural and synthet estrogens in certain animal species increases the frequency of carcinomas of the breast, cervix, vagina, and liver

PREGNANCY CATEGORY X: Estrogens should not be used during pregnancy. See **CONTRAINDICATION** and Boxed Warning.

NURSING MOTHERS: As a general principle, the administration of any drug to nursing mothers should be don only when clearly necessary since many drugs are excreted in human milk.

ADVERSE REACTIONS: The following have been reported with estrogenic therapy; changes in vaginal bleedin pattern and abnormal withdrawal bleeding or flow, breakthrough bleeding, spotting, increase in size of uterin fibromyomata, vaginal candidiasis, change in amount of cervical secretion; tenderness or enlargement of breasts nausea, vomiting, abdominal cramps, bloating, cholestatic jaundice; chloasma or melasma that may persist whe drug is discontinued, erythema multiforme, erythema nodosum, hemorrhagic eruption, loss of scalp hai hirsutism; steepening of corneal curvature, intolerance to contact lenses; headache, migraine, dizziness, menta depression, chorea; increase or decrease in weight; reduced carbohydrate tolerance; aggravation of porphyria edema; changes in libido.

ACUTE OVERDOSAGE: May cause nausea and vomiting.

DOSAGE AND ADMINISTRATION:

PREMARIN® Brand of conjugated estrogens tablets, USP

1. *Given cyclically for short-term use only.* For treatment of moderate-to-severe vasomotor symptoms, atroph vaginitis, or atrophic urethritis associated with the menopause (0.3 mg to 1.25 mg or more daily). The lowest dos that will control symptoms should be chosen and medication should be discontinued as promptly as possible Administration should be cyclic (eg, three weeks on and one week off). Attempts to discontinue or tape medication should be made at three- to six-month intervals.
2. *Given cyclically:* Hypoestrogenism. Osteoporosis.

Hypoestrogenism due to: Female hypogonadism—2.5 mg to 7.5 mg daily in divided doses for 20 day followed by 10 day rest period. If bleeding does not occur by the end of this period, the same dosage schedule i repeated. Female castration or primary ovarian failure—1.25 mg daily, cyclically. Adjust upward or downwar according to response of the patient. For maintenance, adjust dosage to lowest level that will provide effectiv control.

Osteoporosis—0.625 mg daily. Administration should be cyclic (eg, three weeks on and one week off).

PREMARIN® Brand of conjugated estrogens Vaginal Cream

Given cyclically for short-term use only. For treatment of atrophic vaginitis or kraurosis vulvae.

The lowest dose that will control symptoms should be chosen and medication should be discontinued a promptly as possible.

Attempts to discontinue or taper medication should be made at three- to six-month intervals.

Usual dosage range: 2 g to 4 g daily, intravaginally, depending on the severity of the condition.

Patients with an intact uterus who are treated with either PREMARIN Tablets or Vaginal Cream should b monitored for signs of endometrial cancer and appropriate measures taken to rule out malignancy in the event o persistent or recurring abnormal vaginal bleeding.

Revised August 21, 198

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Only standard abbreviations are to be used. Consult the *Council of Biology Editors Style Manual* or the AMA's *Manual for Authors and Editors.* Abbreviations in the title are not acceptable. They should be avoided, if possible, in the abstract. In the text they should be kept to a practical minimum. The full term for which an abbreviation stands should precede its first use in the text unless it is a standard unit of measurement.

Either the generic, chemical, or proprietary names of drugs may be used. If the generic or chemical name is used, authors may, if they desire, insert the proprietary name in parentheses after the first mention in the text, with the name of the manufacturer and city and state.

Regular articles are customarily organized into the following sections: an introduction and headings that identify Material and Methods, Results, and Comment. Authors may wish to summarize their findings in a short paragraph at the end of the Comment section. This format may not be appropriate for some types of articles.

In the introduction, state concisely the purpose and rationale for the study and cite only the most pertinent references as background.

In the *Material and Methods* section describe briefly (but in sufficient detail to permit other workers to evaluate and reproduce the results) the plan, patients and/or experimental animals and controls, methods and procedures utilized, and statistical method(s) employed.

In the *Results* section present the detailed findings. Include mentions of all tables and/or figures. Figures and tables should supplement, not duplicate, the text; presentation of data in either one or the other will suffice. Emphasize only your important observations; do not compare

Estimating length of manuscripts

With the exception of Current Development articles, the length of text material (introduction through Comment section) in regular manuscripts accepted for publication normally ranges from 750 to 4200 words (an average of 2000 words). A text of 4200 words or more can seldom be accepted, especially if tables and figures are included. The average manuscript of 2000 words of text with abstract, 3 tables with captions, 2 figures with legends, and references makes a 5.7-page article in the JOURNAL. The 2000 words of text alone makes approximately 8 pages of manuscript typed double-spaced with the required 1-inch margins (approximately 250 words per page). A table or figure that occupies both columns of half a JOURNAL page is equivalent to approximately 500 words in manuscript. Thus, if a greater number of illustrations and tables is used, the length of the text should be adjusted accordingly.

your observations with those of others. Such comparisons and comments are reserved for the Comment section.

In the *Comment* section state the importance and significance of your findings but do not repeat the details given in the Results section. Limit your opinions to those strictly indicated by the facts in your report. Compare your findings with those of others. No new data should be presented in this section.

Acknowledgments. Acknowledge only persons who have made substantive contributions to the study.

References. A reasonable number (25) is allowed, except in case reports and brief communications (limited to 2) and in manuscripts for the Current Development section (for which there is no limit). Number references consecutively in the order in which they are mentioned in the text. Use the format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" (Vancouver style) (Ann Intern Med 1988;108:258-65). Journal titles should conform to abbreviations used in *Cumulated Index Medicus.*

Examples (if six or fewer authors, list all; if seven or more authors, list three then et al.):

JOURNAL: Flamm BL, Dunnett C, Fischermann E, Quilligan EJ. Vaginal delivery following cesarean section: use of oxytocin augmentation and epidural anesthesia with internal tocodynamic and internal fetal monitoring. AM J OBSTET GYNECOL 1984;148:759-63.

BOOKS: Ledger WJ. Dystocia and prolonged labor. In: Wilson JR, Carrington ER, eds. Obstetrics and gynecology. St. Louis: CV Mosby, 1987 vol 8:474-93.

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For *black-and-white figures,* submit three sets of 3 × 4 inch (minimum) to 5 × 7 inch (maximum) unmounted, glossy prints. All lettering must be done with commercially available paste-on letters (or numbers) or by a professional; typed or freehand lettering is not acceptable. All lettering must be in proportion to the drawing, graph, or photograph. Original drawings, appropriately done in black India ink, roentgenograms, and other material must be submitted as glossy prints with good black-and-white contrast.

For *color figures* submit original transparencies and two sets of unmounted prints on glossy (smooth surface) paper. Polaroid prints are not acceptable. Color transparencies must have a color balance (consistency in lighting and film speed) that is acceptable to the author and Editors before acceptance for publication. Please note that 35 mm transparencies are enlarged to twice their original size. If it is important to deviate from this standard, please so indicate when the material is submitted. The top, first author's last name, and figure number must be indicated on the front of each transparency and the back of each print.

Computer-generated figures must be legible and clearly printed in jet black ink on heavy coated paper with either a glossy or dull finish. Any patterns or shadings must be dark enough for reproduction and must be distinguishable from each other. Lines, symbols, and letters should be both smooth and complete. The legend should not appear on the figure. On the back of each figure the name of the first author and the figure number should be given and the top indicated. Three original individual laser or plotter prints are to be submitted *unmounted* with the manuscript. Laser prints should be full size at 300 dots per inch (DPI) or greater full-page resolution; multiple illustrations on a page cannot be accepted. Dot matrix prints and photographic halftones are not acceptable. *Color figures* are acceptable, but special arrangements must be made with the Editors. The colors used must be dark enough and of sufficient contrast for reproduction. With the exception of fluorescent colors, all colors can be reproduced in four-color figures. The preparation and submitting of color prints should fol-

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Tables. Tables should be typed on separate sheets of paper, one table to a page, and included at the end of the text. They should be numbered in Roman numerals. Each table must be cited in sequence at an appropriate point in the text. Titles should be brief yet indicate clearly the purpose or content of each table, and each column should be precisely defined by headings. Abbreviations and special designations should be explained in a footnote to the table. If a table or any part thereof has been taken from copyrighted material, a footnote to the table must give full credit to the original source. Special arrangements must be made with the Editors for elaborate tables because of space limitations.

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Case reports and brief clinical and basic science communications. Limit of 700 words, 2 references. Include abstract of 50 words maximum, 3 to 5 key words/phrases for indexing purposes, and short title. If tables and/or figures are used, an equivalent number of words must be deducted from the total (see "Estimating Length of Manuscript").

Current Investigation. Same requirements as for regular article.

Clinical Opinion. Limit of 3000 words. Include abstract of 50 to 150 words, 3 to 5 key words/phrases, and short title. Submit to Dr. Zuspan.

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—The original and two copies of the manuscript are submitted.

—The appropriate signed completed checklist and assignment of copyright signed by **all** authors accompany the manuscript.

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—Human experimentation has been approved by the local institution as stated in the Methods section.

—Guidelines for the care and use of animals approved by the institution have been followed as indicated in the Methods section.

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—Three to five key words or short phrases are placed beneath the abstract on page 3.

Tables

- —Each table headed by a title and numbered in Roman numerals is typed double-spaced on a separate page.
- —Tables are cited in sequence in the text.

Figures

- —Each is numbered with an Arabic numeral and cited in numerical sequence in the text.
- —Original transparencies of **color photographs** appropriately numbered and identified are submitted along with two sets of unmounted prints on glossy paper, numbered and identified on the back.

References

- —Are typed double-spaced.
- —Are numbered consecutively in the order they are cited in the text.
- —The format of the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" is used. Examples shown in Information for Authors were followed.
- —Personal communications and unpublished observations are not used as numbered references but are mentioned in the text with the written approval of the person being quoted. The signed approval is enclosed.

AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY

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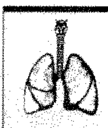
The effects of cocaine abuse have been directly linked to certain congenital anomalies in infants, including intestinal atresia, limb reductions, and brain disruptions. These infants are also at a greater risk for congenital heart, urinary tract, and genitourinary defects.



Approximately 80% of the offspring of heroin-dependent women who receive no prenatal care experience serious medical problems such as intracranial hemorrhages and respiratory distress syndrome.

Neonates exposed to cocaine in utero have been documented to experience neurobehavioral abnormalities. They tend to be irritable and easily overstimulated.

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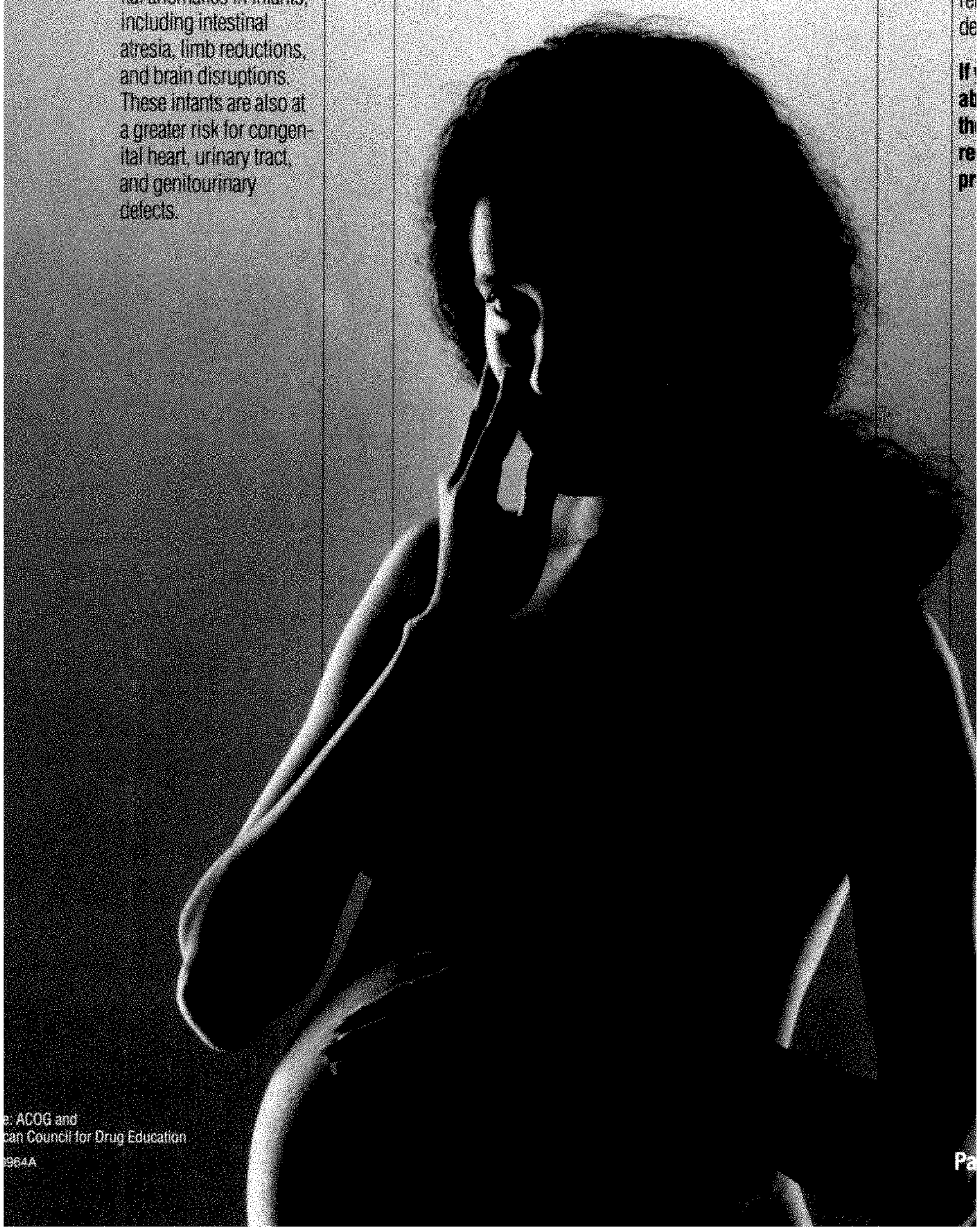

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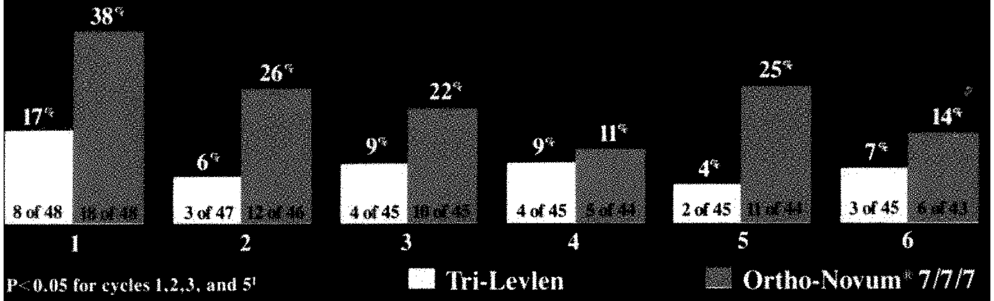
Tri-Levlen means a low incidence of breakthrough bleeding/spotting*†

Multicenter study demonstrates significantly less breakthrough bleeding/spotting through the first six cycles[1]



n = number of women with a bleeding event occurring during a cycle.

TRI·LEVLEN®

Levonorgestrel and ethinyl estradiol tablets · Triphasic regimen

may mean a lot to a woman

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* Incidence defined as percentage of women with a bleeding event occurring at least once during a pill cycle. Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest symptoms of serious disease and discontinue oral contraceptive therapy when appropriate. Please see full prescribing information, a brief summary of which follows.

† Defined as intermenstrual bleeding.

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TRI·LEVLEN® Levonorgestrel and ethinyl estradiol tablets–Triphasic regimen

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BRIEF SUMMARY

Tri-Levlen®—6 brown tablets, each containing 0.050 mg of levonorgestrel (d(-)-13 beta-ethyl-17-alpha-ethinyl-17-beta-hydroxygon-4-en-3-one), a totally synthetic progestogen, and 0.030 mg of ethinyl estradiol (19-nor-17α-pregna-1,3,5(10)-trien-20-yne-3, 17-diol); 5 white tablets, each containing 0.075 mg levonorgestrel and 0.040 mg ethinyl estradiol; 10 light-yellow tablets, each containing 0.125 mg levonorgestrel and 0.030 mg ethinyl estradiol (7 light-green tablets containing inert ingredients are included in the 28-day triphasic regimen).

Indications and Usage

Oral contraceptives are indicated for the prevention of pregnancy in women who elect to use this product as a method of contraception.

Contraindications

Oral contraceptives should not be used in women with any of the following conditions: thrombophlebitis or thromboembolic disorders, a past history of deep-vein thrombophlebitis or thromboembolic disorders, cerebral-vascular or coronary-artery disease, known or suspected carcinoma of the breast, carcinoma of the endometrium or other known or suspected estrogen-dependant neoplasia, undiagnosed abnormal genital bleeding, cholestatic jaundice of pregnancy or jaundice with prior pill use, hepatic adenomas or carcinomas, known or suspected pregnancy.

Warnings

Cigarette smoking increases the risk of serious cardiovascular side effects from oral-contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, gallbladder disease, and hypertension, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this package insert is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long-term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this labeling, epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of disease, namely, a ratio of the incidence of a disease among oral-contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the difference in the incidence of disease between oral-contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods.

1. *Thromboembolic Disorders and Other Vascular Problems*

a. *Myocardial Infarction.* An increased risk of myocardial infarction has been attributed to oral-contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary-artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral-contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral-contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers over the age of 35 and non-smokers over the age of 40 among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in "Warnings"). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors

b. *Thromboembolism.* An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to nonusers to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep-vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease due to oral contraceptives is not related to length of use and disappears after pill use is stopped. A two-to four-fold increase in relative risk of postoperative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate post partum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four to six weeks after delivery in women who elect not to breast-feed, or a midtrimester pregnancy termination.

c. *Cerebrovascular diseases.* Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and nonusers, for both types of strokes, while smoking interacted to increase the risk for hemorrhagic strokes. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for nonsmokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women.

d. *Dose-related risk of vascular disease from oral contraceptives.* A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high-density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high-density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the nature and absolute amount of progestogen used in the contraceptive. The amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral-contraceptive agents should be started on preparations containing less than 50 mcg of estrogen.

e. *Persistence of risk of vascular disease.* There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral-contraceptive formulations containing 50 micrograms or higher of estrogens.

2. *Estimates of Mortality from Contraceptive Use.* One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral-contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is less than that associated with childbirth. The observation of a possible increase in risk of mortality with age for oral-contraceptive users is based on data gathered in the 1970's—but not reported until 1983. However, current clinical practice involves the use of lower estrogen dose formulations combined with careful restriction of oral-contraceptive use to women who do not have the various risk factors listed in this labeling. Because of these changes in practice and, also, because of some limited new data which suggest that the risk of cardiovascular disease with the use of oral contraceptives may now be less than previously observed, the Fertility and Maternal Health Drugs Advisory Committee was asked to review the topic in 1989. The Committee concluded that although cardiovascular-disease risks may be increased with oral-contraceptive use after age 40 in healthy nonsmoking women (even with the newer low-dose formulations), there are greater potential health risks associated with pregnancy in older women and with the alternative surgical and medical procedures which may be necessary if such women do not have access to effective and acceptable means of contraception. Therefore, the Committee recommended that the benefits of oral-contraceptive use by healthy nonsmoking women over 40 may outweigh the possible risks. Of course, older women, as all women who take oral contraceptives, should take the lowest possible dose formulation that is effective.

3. *Carcinoma of the Reproductive Organs.* Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. The overwhelming evidence in the literature suggests that use of oral contraceptives is not associated with an increase in the risk of developing breast cancer, regardless of the age and parity of first use or with most of the marketed brands and doses. The Cancer and Steroid Hormone (CASH) study also showed no latent effect on the risk of breast cancer for at least a decade following long-term use. A few studies have shown a slightly increased relative risk of developing breast cancer, although the methodology of these studies, which included differences in examination of users and nonusers and differences in age at start of use, has been questioned. Some studies suggest that oral-contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. In spite of many studies of the relationship between oral-contraceptive use and breast and cervical cancers, a cause-and-effect relationship has not been established.

4. *Hepatic Neoplasia.* Benign hepatic adenomas are associated with oral-contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use. Rupture of rare, benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral-contraceptive users. However, these cancers are extremely rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral-contraceptive users approaches less than one per million users.

5. *Ocular Lesions.* There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia; papilledema; or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately.

6. *Oral-Contraceptive Use Before or During Early Pregnancy.* Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. Studies also do not suggest a teratogenic effect, particularly insofar as cardiac anomalies and limb-reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods, pregnancy should be ruled out before continuing oral-contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral-contraceptive use should be discontinued if pregnancy is confirmed

7. *Gallbladder Disease.* Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral-contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral-contraceptive formulations containing lower hormonal doses of estrogens and progestogens

8. *Carbohydrate and Lipid Metabolic Effects.* Oral contraceptives have been shown to cause glucose intolerance in a significant percentage of users. Oral contraceptives containing greater than 75 micrograms of estrogens cause hyperinsulinism, while lower doses of estrogen cause less glucose intolerance. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the nondiabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects, prediabetic and diabetic women should be carefully observed while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see "Warnings" 1a. and 1d.), changes in serum triglycerides and lipoprotein levels have been reported in oral-contraceptive users.

9. *Elevated Blood Pressure.* An increase in blood pressure has been reported in women taking oral contraceptives and this increase is more likely in older oral-contraceptive users and with continued use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing quantities of progestogens. Women with a history of hypertension or hypertension-related diseases, or renal disease should be encouraged to use another method of contraception. If women with hypertension elect to use oral contraceptives, they should be monitored closely, and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension among ever-and never-users.

10. *Headache.* The onset or exacerbation of migraine or development of headache with a new pattern that is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause.

11. *Bleeding Irregularities.* Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. The type and dose of progestogen may be important. Nonhormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding, as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent.

Precautions

1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed, persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. (See "Warnings" 1d.) 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Patients becoming significantly depressed while taking oral contraceptives should stop the medication and use an alternate method of contraception in an attempt to determine whether the symptom is drug related. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact-lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine-and liver-function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X, decreased antithrombin 3; increased norepinephrine-induced platelet aggregability. b. Increased thyroid-binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radioimmunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral-contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See "Warnings" section. 10. PREGNANCY. Pregnancy Category X. See "Contraindications" and "Warnings" sections. 11. NURSING MOTHERS. Small amounts of oral-contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child

Information for the Patient

See Patient Labeling.

Adverse Reactions

An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see "Warnings" section): thrombophlebitis, arterial thromboembolism, pulmonary embolism, myocardial infarction, cerebral hemorrhage, cerebral thrombosis, hypertension, gallbladder disease, hepatic adenomas or benign liver tumors.

There is evidence of an association between the following conditions and the use of oral contraceptives, although additional confirmatory studies are needed: mesenteric thrombosis, retinal thrombosis.

The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug related: nausea, vomiting; gastrointestinal symptoms (such as abdominal cramps and bloating); breakthrough bleeding; spotting; change in menstrual flow; amenorrhea; temporary infertility after discontinuation of treatment; edema; melasma which may persist; breast changes: tenderness, enlargement, secretion; change in weight (increase or decrease); change in cervical erosion and secretion; diminution in lactation when given immediately postpartum; cholestatic jaundice; migraine; rash (allergic); mental depression, reduced tolerance to carbohydrates; vaginal candidiasis; change in corneal curvature (steepening); intolerance to contact lenses.

The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: congenital anomalies, premenstrual syndrome, cataracts, optic neuritis, changes in appetite, cystitis-like syndrome, headache, nervousness, dizziness, hirsutism, loss of scalp hair, erythema multiforme, cerebral-vascular disease with mitral valve prolapse, lupus-like syndromes, erythema nodosum, hemorrhagic eruption, vaginitis, porphyria, impaired renal function, hemolytic uremic syndrome, Budd-Chiari syndrome, acne, changes in libido, colitis, sickle-cell disease.

Overdosage

Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea, and withdrawal bleeding may occur in females.

Noncontraceptive Health Benefits

The following noncontraceptive health benefits related to the use of oral contraceptives are supported by epidemiological studies which largely utilized oral-contraceptive formulations containing doses exceeding 0.035 mg of ethinyl estradiol or 0.05 mg of mestranol.

Effects on menses: increased menstrual cycle regularity, decreased blood loss and decreased incidence of iron-deficiency anemia, decreased incidence of dysmenorrhea.

Effects related to inhibition of ovulation: decreased incidence of functional ovarian cysts, decreased incidence of ectopic pregnancies.

Effects from long-term use: decreased incidence of fibroadenomas and fibrocystic disease of the breast, decreased incidence of acute pelvic inflammatory disease, decreased incidence of endometrial cancer, decreased incidence of ovarian cancer.

Dosage and Administration

To achieve maximum contraceptive effectiveness, TRI-LEVLEN® tablets (Levonorgestrel and Ethinyl Estradiol Tablets—Triphasic Regimen) must be taken exactly as directed and at intervals not exceeding 24 hours.

For full details on dosage and administration see prescribing information in package insert. 60627-0 5/90

Reference: 1. Data on file, Berlex Laboratories.

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CLINICAL SECTION

Clinical Opinion

Screening recommendations for gestational diabetes mellitus

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As a result of extensive experiences in multiple centers and a review of the current literature, we conclude that a plasma glucose level obtained 1 hour after a 50 gm oral glucose challenge is the "best" gestational diabetes mellitus screening test. This universal screening is performed at least once during pregnancy. The screening threshold should be no higher than 140 mg/dl, or an unacceptable loss in sensitivity occurs. Universal screening for gestational diabetes mellitus is justified by morbidity reduction, cost, and protocol simplicity and ease. (AM J OBSTET GYNECOL 1991;165:493-6.)

Key words: Gestational diabetes, screening

Gestational diabetes mellitus is the most common metabolic complication that affects pregnant women. The frequency of gestational diabetes mellitus and its associated maternal, perinatal, and long-term morbidity emphasize the importance of an appropriate screening method. The clinician who attempts to determine how best to screen patients for gestational diabetes mellitus is confronted with conflicting information and recommendations. This article summarizes the information that forms the basis for the authors' recommendations about screening for gestational diabetes mellitus.

Significance of gestational diabetes mellitus

Gestational diabetes mellitus is defined as carbohydrate intolerance with onset or recognition during pregnancy.[1] It is important to identify a pregnant woman with gestational diabetes mellitus because gestational diabetes mellitus is common and is associated with significant metabolic alterations, increased perinatal and maternal morbidity, and exaggerated long-term morbidity among the mothers and their offspring.

Perinatal mortality is significantly increased in gestational diabetic pregnancies if the metabolic abnormality is not recognized or if it is recognized but not treated properly.[2] In addition to an increased risk of intrauterine fetal death, commonly reported perinatal morbidity associated with gestational diabetes mellitus includes an increased incidence of macrosomia, birth trauma, neonatal hypoglycemia, hyperbilirubinemia, hypocalcemia, and polycythemia.[3] Although recent studies report enviable results regarding perinatal morbidity in cases of gestational diabetes mellitus[4, 5] there is evidence that maternal and perinatal morbidity and mortality is significantly increased in women with gestational diabetes mellitus when compared with nondiabetic pregnant women.[6, 7] Short-term maternal morbidity includes an increased incidence of chronic hypertension, hydramnios, and cesarean section.[7]

Gestational diabetes mellitus also has long-term morbidity implications for the mother and her offspring. Data from human and animal studies suggest that female fetuses exposed to chronic hyperglycemia may suffer subclinical islet cell injury.[8] Women with gestational diabetes mellitus have an increased likelihood of remaining carbohydrate intolerant after pregnancy,[9] a significantly increased risk of type II (non-insulin-dependent) diabetes later in life, and a predisposition to

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obesity, hyperlipidemia, atherosclerotic vascular disease, and increased systolic blood pressure and mortality.[10] The offspring of women with gestational diabetes mellitus have a higher incidence of childhood obesity,[11] type II diabetes during childhood and later in life,[11] and intellectual and motor impairment.[12]

Screening methods for gestational diabetes mellitus

A variety of methods have been proposed to screen for gestational diabetes mellitus. The Second International Workshop-Conference recommended screening with a 50 gm oral glucose load followed 1 hour later by a glucose determination in blood.[1] A recent survey has confirmed that the 1-hour oral 50 gm glucose challenge is the preferred method of screening for gestational diabetes mellitus.[13] Ninety-six percent of maternal-fetal medicine subspecialists and 84% of fellows of the American College of Obstetricians and Gynecologists (ACOG) use this method in their practice. The 50 gm oral glucose load may be administered to patients in the fasting or fed state.[14]

A 1-hour period is more efficient for screening than a 2-hour window, but it has been suggested that a 2-hour blood determination may improve specificity.[15] A 2-hour postprandial plasma glucose level has been suggested for screening nonpregnant individuals for diabetes. Random blood glucose screening seems desirable because it does not inconvenience patients and might be more cost-efficient. Lind[16] has determined 99% cutoff values of 100 mg/dl within 2 hours of a meal compared with 101 mg/dl >2 hours after a meal. However, few women with gestational diabetes mellitus have been identified by means of 2-hour postprandial or random glucose levels, so that appropriate cutoff values have not been established; therefore it has not been possible to determine the clinical utility of these methods. Glycosylated serum protein levels have been correlated with glycemic control in nonpregnant individuals, which led investigators to examine whether such tests might be effective in screening for gestational diabetes mellitus. Glycosylated hemoglobin measurements have been most studied, and they do not appear to be helpful in the detection of gestational diabetes mellitus.[17] When compared with glycosylated hemoglobin levels, the 1-hour 50 gm glucose challenge had greater specificity, sensitivity, and predictive value for a positive diagnosis.[17] Similarly, neither glycosylated albumin nor protein[18] has been of value in the diagnosis of gestational diabetes mellitus.

In view of the preceding information we recommend screening for gestational diabetes with a plasma glucose level 1 hour after a 50 gm oral glucose load.

Sensitivity and specificity considerations

An ideal screening test should have high sensitivity (proportion of cases with the disease under consideration correctly identified) and high specificity (proportion of patients without the disease correctly identified).

The choice of a threshold for screening is based on the degree of sensitivity desired and the amount of specificity to be sacrificed. In gestational diabetes mellitus this translates into the number of 3-hour 100 gm oral glucose tolerance tests one is willing to perform to detect a given proportion of gestational diabetes mellitus cases in the population. To accurately assess these test functions an ideal study would include both the screening test and the gold standard diagnostic test on all subjects. Only the study of O'Sullivan et al.[19] fulfills these criteria. In that study of 752 pregnant women, a venous whole-blood glucose level >130 mg/dl that was obtained 1 hour after a 50 gm glucose challenge was 79% sensitive and 87% specific for gestational diabetes mellitus. In a population-based study of 6214 pregnant women[20] evaluated by venous plasma and the hexokinase method of glucose analysis, the diagnostic test was performed if the 50 gm 1-hour screening value was >130 mg/dl. Although it is possible that cases of gestational diabetes went undetected because of screening values <130 mg/dl, it was assumed that sensitivity was 100% at this level. If a screening test threshold of 140 mg/dl had been used, 10% of cases would have been missed (90% sensitivity). Similar results have been reported by other investigators,[21] who also noted that gestational diabetic women with screening test values between 130 and 139 mg/dl were at significant risk for maternal hyperglycemia and neonatal hypoglycemia and macrosomia. If these data[20] are used, 14% of pregnant women would require a 3-hour oral glucose tolerance test (86% specificity) to achieve 90% sensitivity, although in both of the studies cited earlier[20, 21] 23% would require oral glucose tolerance tests (77% specificity) to achieve 100% sensitivity. Thus the obstetrician is faced with the choice of whether to use a threshold of 140 mg/dl and miss the diagnosis in 10% of the women with gestational diabetes to perform oral glucose tolerance tests on only 14% of the pregnant population or to use a threshold of 130 mg/dl and detect nearly all of the cases of gestational diabetes but perform oral glucose tolerance tests in 23% of the pregnant population. Making such a decision requires consideration of available economic resources and laboratory logistics.

As a result of this information, we recommend the use of a screening threshold no higher than 140 mg/dl.

Cost-effectiveness considerations

It is worthwhile to consider the cost of screening protocols for gestational diabetes mellitus. Although the morbidity implications and potential benefits of diagnosing gestational diabetes mellitus have been discussed previously, it is difficult (if not impossible) to allocate a dollar value to these variables. For this reason most investigators have started with this assumption made by the ACOG, the American Diabetes Association, and other groups: There are benefits for patients in whom gestational diabetes mellitus is diagnosed. Reports have focused on the relative cost of diagnosing gestational diabetes mellitus in various segments of the obstetric population. Because the prevalence of gestational diabetes mellitus increases with advancing maternal age[19, 20] and because the presence of other risk factors increases the likelihood of gestational diabetes mellitus, age and other risk factors are considered when patients are selected for screening.[22] Although one study demonstrated a similar cost per case diagnosed among patients with or without risk factors for gestational diabetes, another study suggested that screening only women aged ≥24 years would yield a cost per case diagnosed that is approximately 50% less than that of universal screening. In a population-based study[20] of 125 cases of gestational diabetes mellitus among 6214 pregnant women, sensitivity went from 65% to 85% when the age cutoff was decreased from >30 years (the current ACOG screening recommendation) to >25 years, while the cost per case diagnosed increased by only 1%! Thus no advantage is gained from a maternal age threshold for screening of 30 years, and the threshold could be decreased to 25 years with no increase in cost.

Screening patients exclusively on the basis of age fails to consider other risk factors. For instance, an obese 25-year-old gravid patient with a family history of diabetes has a risk of gestational diabetes mellitus equivalent to that of a nonobese 34-year-old woman without a family history.[22] Furthermore, gestational diabetes mellitus of a severity that poses increased risk of perinatal morbidity and mortality is likely to be found among both young and older gravid patients.[22] Ethnicity also affects the incidence of gestational diabetes. For example Navajo Indian women, Mexican women, women of Indo-Chinese descent, Chinese women with high prepregnancy ponderal indices, Saudi Arabian women, and black and Mexican teenagers have increased rates of gestational diabetes mellitus.

The issues of frequency and timing of glucose testing during pregnancy are linked to the issues of reliability of the individual tests and metabolic changes that are dependent on gestational age. Existing data[22, 23] suggest that glucose intolerance increases as gestation advances. Women with elevated glucose levels at screening and normal glucose tolerance test results early in pregnancy are at increased risk for the development of gestational diabetes mellitus later in pregnancy when compared with patients who have initially normal screening values.[22] Sequential screening, glucose tolerance testing,[23] or both[22] increase the yield of gestational diabetes mellitus identifications.

Universal screening for gestational diabetes mellitus, without regard to maternal age, is the most appropriate protocol. This recommendation is based on two lines of reasoning. The first is that gestational diabetes mellitus should be diagnosed because of the potentially preventable morbidity and mortality associated with this problem. In addition, adding complexity to test administration and interpretation leads to patient and physician error and noncompliance.[24] In the population-based study previously described,[20] 22% of pregnant women were under the age of 25 and had no risk factors for gestational diabetes; thus they would not have been screened. If a protocol requires historic risk factor evaluation, a decision must be made for each patient. Each decision point increases the possibility of error and takes the physician's time to consider the risk(s) and to explain to each patient why she does nor does not need screening. It would be more efficient and less error prone to use universal screening, in which a clerk would schedule the test automatically at 24 to 28 weeks' gestation and would provide each patient with a written explanation.

As a result of our study information, we recommend screening all pregnant women for gestational diabetes mellitus at least once during pregnancy. Testing between 24 and 28 weeks' gestation is late enough to demonstrate glucose intolerance but early enough to allow appropriate interventions. Patients with gestational diabetes mellitus risk factors may benefit from earlier screening. Patients with initially elevated screens that are followed by normal glucose tolerance tests may benefit from repeat testing later in pregnancy.

Oral glucose tolerance testing methods

If a patient's 1-hour 50 gm glucose screening level shows abnormality, she should be tested with the definitive diagnostic test, a 100 gm 3-hour glucose tolerance test.[1, 19] The patient is instructed to consume at least 150 mg of carbohydrate daily for the 3 days before the oral glucose tolerance test. The patient who is to have a 3-hour oral glucose tolerance test should take nothing by mouth overnight for at least 8 to 10 hours before the test.[1, 25] On the morning of the test the patient may drink water but should not ingest caffeine or food or liquid containing calories. It is also best that the patient not smoke for 12 hours before the test. Ideally, the patient should rest for approximately 30 minutes before the test. During that time period she may read, listen to the radio, and/or converse, but she should not move around. A heparin lock may be placed to avoid repeated needle sticks.

After the fasting sample is drawn, the patient drinks 100 gm of a glucose solution. For consistency, the timing clock is started as the patient begins to drink. The drinking should be completed within 5 minutes.[25] Subsequent blood samples are taken 1, 2, and 3 hours after the patient has drunk the glucose solution. During that time the patient should not move around or smoke.

When the test has been completed, the patient may eat or drink to minimize rebound hypoglycemia.

The rigorousness of the preceding methods does not apply when the patient has a 1-hour 50 gm glucose screen. Then the patient need not fast, be prepared by purposeful rest or diet before the test or be purposely isolated from activity. If the patient is a smoker, it is preferable that she not smoke a cigarette within 2 hours before the test.

In summary, we recommend: (1) Universal screening of prenatal patients for gestational diabetes mellitus should be done at least once during pregnancy. All prenatal patients should be screened, regardless of age, ethnicity or the presence of other risk factors for diabetes. (2) A 1-hour plasma glucose level after a 50 gm oral glucose challenge should be used for screening. (3) A screening threshold level no higher than 140 mg/dl should be used. (4) A "positive" screen should be followed up with the diagnostic 3-hour oral glucose tolerance test.

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Additional references are available from Dr. Cousins on request.

Embryonic ultrasonographic measurements: Crown-rump length revisited

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New endovaginal probes will result in many investigators reexamining the crown-rump length data first generated with static arm and abdominal real-time scanners. As new tables are produced, only through better understanding of the anatomy of early embryos can we realize what we are actually seeing and measuring (or at least attempting to measure). Furthermore, as we try to correlate early embryonic size with gestational age, we realize that the crown-rump measurement is not applicable in principle or in nomenclature when the embryo is <18 mm long. When first seen, the embryo is relatively straight; a measurement of its size is best described as "greatest length." As the embryo curves into a C-shaped, tadpolelike structure, the greatest measurement along the long axis is actually a neck-rump measurement. Further unfolding of the head and regression of the tail finally allow an accurate crown-rump measurement (at about 18 mm) as we have used it for almost two decades. Early embryonic stages and characteristics are reviewed and methods of measurement of early embryonic size are explained. (AM J OBSTET GYNECOL 1991;165:497-501.)

Key words: Crown-rump length, embryology, ultrasonography, endovaginal ultrasonography, early embryonic size

In 1973 Robinson[1] introduced a new approach to the problem of assessing embryonic maturity during the first trimester of pregnancy by devising a technique for the ultrasonographic measurement of fetal crown-rump length. He provided a step-by-step description of how to take measurements with the static arm scanners that were the standard of the day.[2] Although the regression curve[3] is usually reproduced back to a menstrual age of 6 weeks 2 days (with a predicted crown-rump length of 6.7 mm), Robinson states that "when the crown-rump length is below 12 mm the technique described is not entirely applicable as the fetal length is at this time only marginally greater than its breadth."[1] This limitation of the technique was experienced by other authors performing studies with static arm scanners. The smallest mean crown-rump length reported by Drumm et al.[4] was 10.8 mm.

The widespread use of real-time ultrasonographic equipment made it technically easier to take such measurements. More investigators examined this area, but

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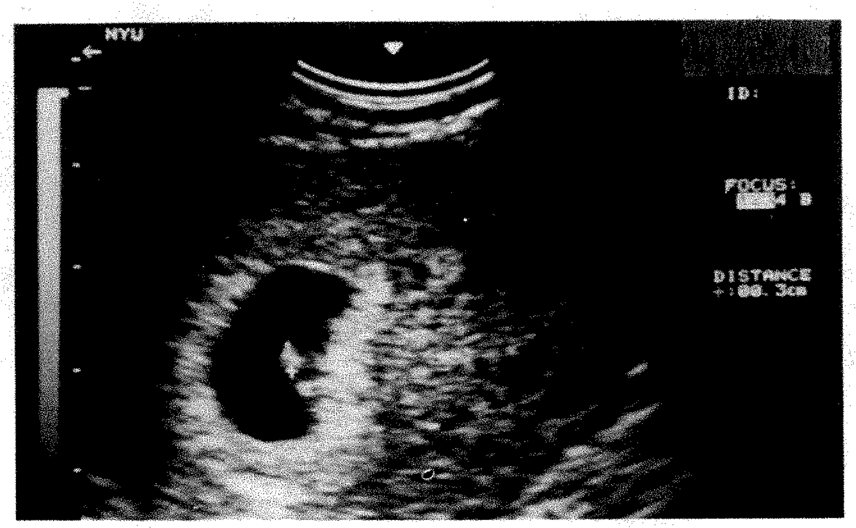

Fig. 1. Highly magnified endovaginal scan of gestation 44 days after mother's last menstrual period. Note 3 mm embryonic thickening adjacent to yolk sac contained within gestational sac.

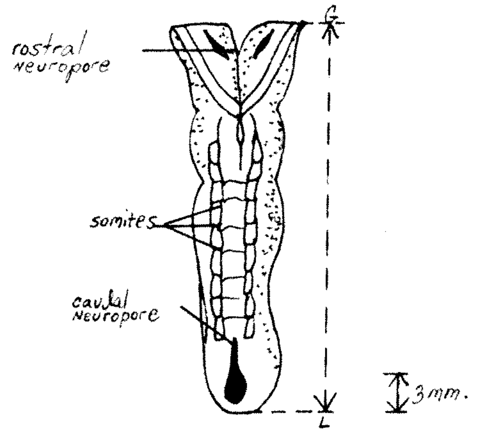

Fig. 2. This 3 mm embryonic structure clearly shows rostral and caudal neuropores. Embryo is fairly straight; measurement of its size is actually its greatest length *(G—L)*.

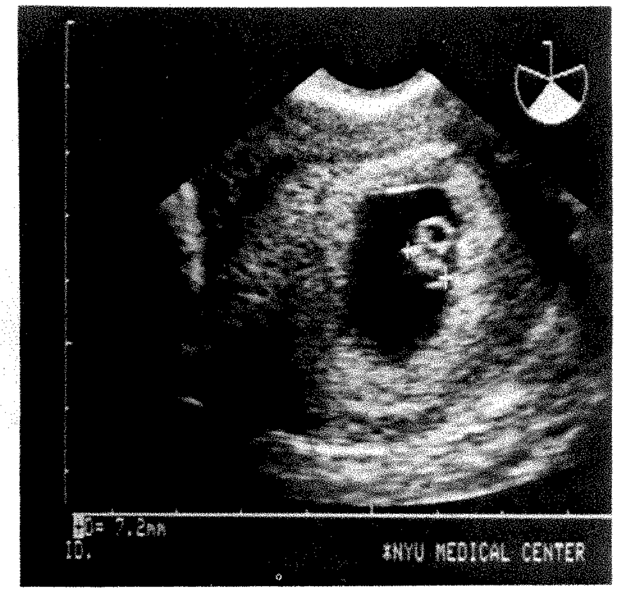

Fig. 3. Embryonic structure measuring 7.2 mm 50 days after mother's last menstrual period is well visualized in this gestational sac. Note again embryo adjacent to yolk sac.

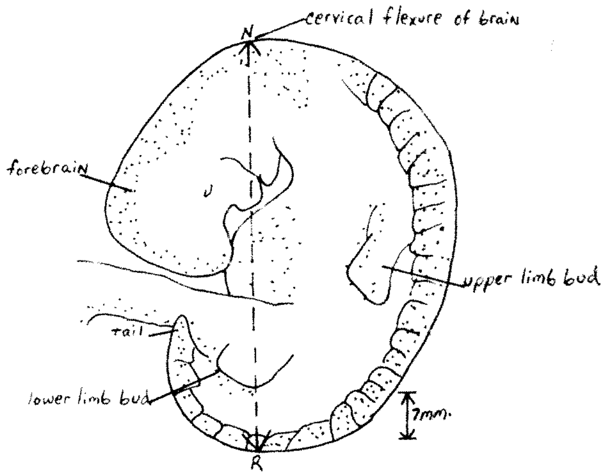

Fig. 4. Note C-shape assumed by this embryo of corresponding gestational age to embryo shown in Fig. 3. Forebrain has developed from rostral neuropore. Tail has developed caudally. Upper and lower limb buds are visible. Greatest measurement of embryonic size along long axis is 7 mm and represents neck-rump *(N—R)* measurement.

their findings were relatively similar.[5-7] A subsequent study of ovulation-timed pregnancies suggested that previous dating curves had underestimated gestational age.[8] Although the actual data points were not supplied, the graph and charts generated began with a crown-rump length of 10 mm.

The advent of new endovaginal probes provides such a degree of magnification as to be a form of "ultrasonomicroscopy."[9] It is as if we were doing ultrasonographic examinations through a low-powered microscope, imaging structures that we would not be able to see with the naked eye if those structures were held at arm's length. This new technology initially generated great interest in early pregnancy detection.[10] Now attention is turned to crown-rump length. Because we are able to image embryonic structures as soon as they appear distinct from the yolk sac, we must reevaluate the anatomy of what we are seeing and measuring ultrasonographically.

Historical background

Interest in establishing standard measurements for embryos dates back at least to the work by Mall[11] first published in 1918. He noted that the "main measurements employed were sitting-height and standing-height; these are known respectively as crown-rump and crown-heel."[11] Further, he noted that "it is well known that embryologists are given to using the crown-rump measurement for smaller specimens, while anthropologists and obstetricians generally use the crown-heel measurement for larger specimens."[11] The embryo's developmental stages, characteristics, and sizes were studied by embryologists.[12, 13] Early investigators of ultrasonographic measurements of crown-rump length[1, 4] made frequent reference to the embryologic staging and development of early embryos, even explaining why some of their data differed.

Embryologic stages

The distinction between embryo (from the Greek, meaning *metamorphosis*) and fetus (from the Latin, meaning *offspring*) is somewhat arbitrary. By 9 days after conception the embryonic complex, consisting of the primary yolk sac, embryonic disk, and amniotic cavity, is present.[14] Imaging the embryonic complex at this

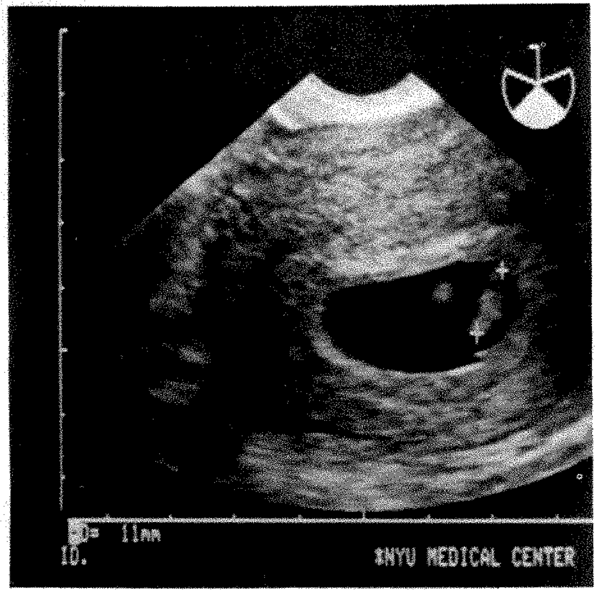

Fig. 5. Embryo measuring 11 mm at 55 days after mother's last menstrual period. See Fig. 6.

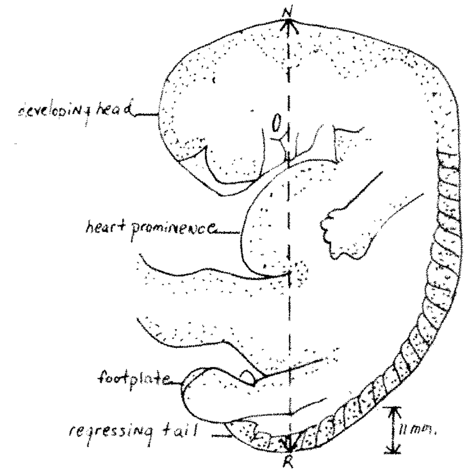

Fig. 6. Further development of head and its extension. Tail is regressing. Measurement along longest axis is from rump to cervical flexure (neck).

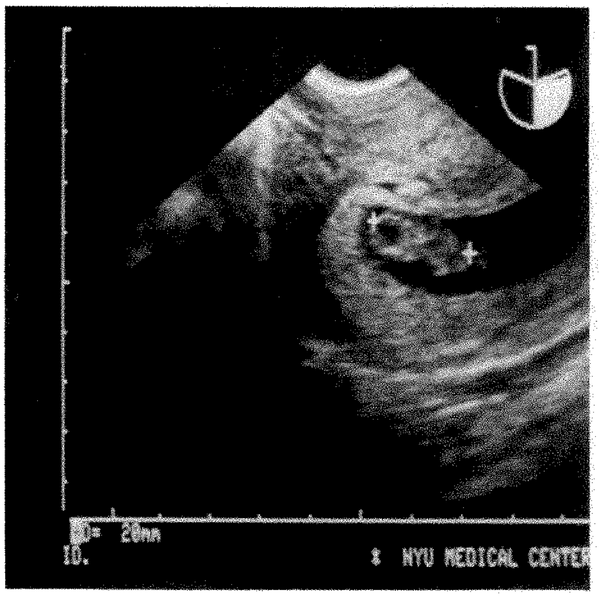

Fig. 7. Vaginal sonogram of embryo at 62 days after mother's last menstrual period. Crown-rump length is 20 mm. Sonolucent portion within head is developing rhombencephalon.

stage, however, is well beyond the capability of even the "sonomicroscopy" of our current vaginal probes. As gestation progresses, the first structure imaged within the gestational sac by ultrasonography is the yolk sac. The embryo and its cardiac activity are present, but they are still too small to be imaged (although with M-mode echocardiography a cardiac signal can occasionally be detected adjacent to the yolk sac).[15] Further embryonic growth is first noticed ultrasonographically as a thickening along the yolk sac. The following abbreviated description of embryonic stages and their appearance is meant to enhance understanding of the anatomy so that we realize what we are measuring (or at least striving to measure) ultrasonographically. The nomenclature needs to be revised: Crown-rump length is a convenient term, but when the embryo is <18 mm we are not really measuring crown-rump length.

Until the embryo reaches 3 to 4 mm in length it is straight (Figs. 1 and 2); instead of measuring crown-rump length, we are actually measuring greatest length.[16] The neural tube has formed opposite the somites. Rostral and caudal neuropores occupy the two distal portions. As the embryo grows, it curves until it is almost C-shaped (Figs. 3 and 4). The rostral neuropore closes, develops into the forebrain prominence, and becomes the head. The caudal neuropore elongates into a tail. The primitive heart still has great prominence. The greatest length along the longest axis begins to give way to a neck-rump measurement. This is the result of the extreme flexion of the developing head. With further development the tail regresses, the limb buds develop into hands and feet, the head begins to extend, and the heart remains prominent. The neck-rump length is now about 11 mm (Figs. 5 and 6). It is not until the greatest long-axis measurement reaches 18 to 22 mm that we approach the true crown-rump length measurement as we have previously understood it. The further unfolding of the head, the final regression of the tail, the development of the limbs and eyes, and the regression of the heart prominence allow what was previously a tadpolelike form to now be recognizable as an offspring (fetus) (Figs. 7 and 8).

When the crown-rump length reaches 35 mm (Figs.

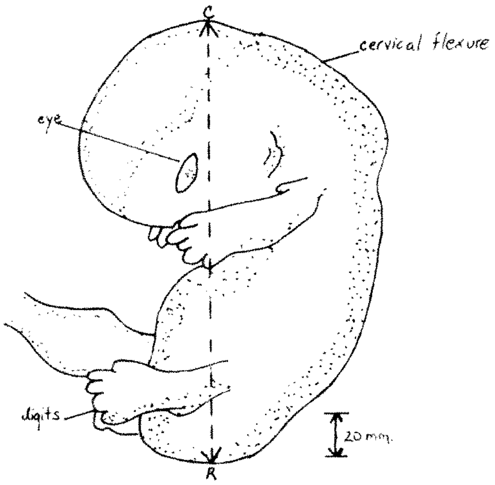

Fig. 8. Further extension and development of head and further regression of tail allow longest length of this embryo to be measured from crown to rump *(C—R)*. Note location of cervical flexure at this stage relative to its location in Figs. 6 and 4.

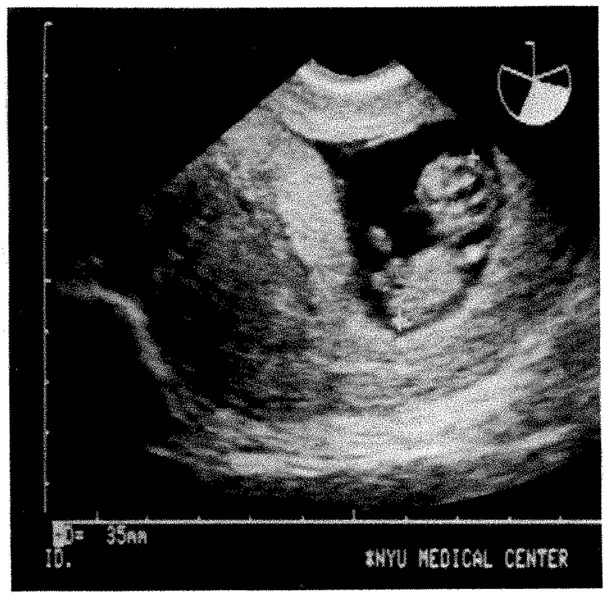

Fig. 9. Vaginal sonogram of a fetus at 72 days after mother's last menstrual period. Crown-rump length is 3.5 cm. Head, trunk, and limbs are readily recognizable.

9 and 10), even the most unsophisticated observers (the patient and her husband) can recognize the "baby," and the ultrasonographer is confident that the landmarks necessary for an accurate measurement are easily identifiable. This will remain true until the continued flexion of the fetus and the increasing ease of taking early biparietal diameter measurements cause the parameter of choice to switch from measurement along the long axis of the sitting embryo to measurement of the biparietal diameter.

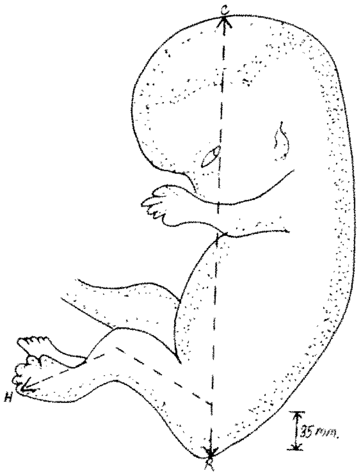

Fig. 10. This drawing corresponds to sonogram seen in Fig. 9. Crown-rump length is 35 mm. Distance from crown to heel *(C—H)* would actually represent standing height of fetus, as opposed to sitting height (crown-rump length).

Implications for crown-rump length charts to come

Experienced ultrasonographers have long realized that "one should use the average crown-rump length measurement from three satisfactory images."[17] This will not be difficult to do with confidence as the embryo enlarges (Figs. 8 and 10). However, even with the best of efforts, the measurements in very small embryos may be somewhat tangential and may not truly represent the greatest length, causing gestational age to be inaccurately estimated. This problem is similar to the problems initially encountered in measuring femur length. If great care to find appropriate landmarks is not taken, a slightly tangential view will show a deceptively short length. As new studies of early embryonic size are done, tangential views of very small embryos when compared with menstrual data (no matter how reliable the dates) will give a deceptively small *mean* embryonic size for a given menstrual age. Therefore maximum image magnification and the anatomic landmarks outlined should be used. When early embryonic size exceeds 18 mm and becomes a true crown-rump length (no longer a greatest length or neck-rump length), the likelihood of deceptively low measurements decreases.

Implications for crown-rump length charts currently in use

Investigators[4, 17, 18] have concluded that the original crown-rump length data by Robinson[1] remain the most reliable. The smallest crown-rump length image published from his work is 12 mm.[2] It is assumed that this represents the best of his available early images. None of the early crown-rump length observers mention the yolk sac; it was not yet ultrasonographically noticeable. It is conceivable that some of the very small crown-rump length measurements included a portion of yolk sac. Robinson himself was concerned about reliability of measurements <12 mm.

Measurements of embryonic size that truly represent crown-rump length (>18 mm) will still prove to be reliable even though the measurements originated in the static arm era. I believe that new and more sophisticated equipment and enhanced understanding of embryologic anatomy will enable us to construct dating charts of early embryonic size, taking the greatest measurement on the long axis. Because this goes from greatest length to neck-rump length and finally a true crown-rump length, it may be preferable to only use a generic term such as early embryonic size when describing embryonic length <18 mm.

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Ovarian cancer screening: Potential effect on mortality

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Serum tumor markers and ultrasonography are being investigated as possible ovarian cancer screening tests. Data from the United States on ovarian cancer incidence and survival were used to estimate the potential benefit on ovarian cancer mortality from screening tests of various sensitivities. A test with 80% sensitivity could reduce ovarian cancer mortality by 50% if all screening-detected cases were to experience current stage I survival rates; the benefit would be greatest among women aged 45 or older. For each cancer detected there would be at least 50 false-positive screening tests unless test specificity is >98%. If our most optimistic assumptions about screening could be met, then universal periodic screening of women aged 45 to 74 would result in about 5000 additional 5-year survivors of ovarian cancer annually. Uptake of existing screening tests is far less than universal; thus we would expect the impact of any ovarian cancer screening program to fall short of these projections. (Am J Obstet Gynecol 1991;165:502-5.)

Key words: Ovarian cancer screening, ovarian cancer mortality

Screening for early detection of certain cancers in women has proved effective. The Papanicolaou smear is credited with reducing mortality rates from cervical cancers,[1] and mammography is leading to earlier detection of breast cancer, although the use of mammography has yet to translate into a decrease in mortality in the general population.[2,3] Gynecologists are now considering whether deaths that result from ovarian cancer also might be prevented by the identification of and use of a screening test for this disease. Many investigators in the United States and Europe are evaluating the use of modalities such as urine or serum tumor markers and ultrasonographic imaging for the early detection of ovarian cancer.[4-8] Public belief in the value of screening is leading to a demand for these tests before their value has been demonstrated.

This article examines the reduction in ovarian cancer mortality that might be achieved if we can develop a screening test for the detection of this disease during its preclinical phase. We also consider how many screening tests would have to be performed to gain each additional survivor, how many false-positive tests would occur, and how large a study would be required to evaluate a mortality reduction that resulted from screening.

Methods

Calculating the effect of screening on ovarian cancer mortality in the United States required estimates of the number of cases and deaths from ovarian cancer by age and stage at diagnosis. These were extrapolated from data collected from 1973 to 1986 by means of a public-use data tape of the Surveillance, Epidemiology and End Results (SEER) program of the National Cancer Institute. Age-specific, stage-specific 5-year ovarian cancer survival rates are based on the experience of women with follow-up in SEER registries from 1973 to 1980.[9] Additional data regarding survival rates for stage IA and IB disease were taken from a randomized trial of treatment.[10]

To estimate the effect of a screening test on survival, we assumed that women whose cancers were detected by screening would experience survival rates equal to or better than those currently experienced by women in the same age group in whom diagnosis was made at stage I. We varied the sensitivity of our hypothetical screening test from 70% to 90% because this is comparable to the best sensitivities reported for Papanicolaou smears[11] and mammography.[12] Sensitivity is the percentage of test results that are positive among women with the disease. We assumed that cases detected and cases missed by screening have the same age and stage distribution and the same mortality in the absence of screening.

The main outcome in this analysis is the number of

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Table I. Estimated annual United States ovarian cancer cases by age and stage (extrapolated from SEER, 1973 to 1986

Age (yr)	*Stage I (No.)*	*Stage II (No.)*	*Stage III-IV (No.)*	*Unstaged (No.)*	*All stages (No.)*
<25	311	68	172	31	582
25-34	471	85	283	32	871
35-44	611	164	755	85	1,615
45-54	1,023	464	1,920	167	3,574
55-64	1,101	682	3,164	239	5,186
65-74	732	586	2,946	306	4,570
≥75	475	442	2,154	465	3,536
ALL	4,724	2,491	11,394	1,325	19,934

Table II. United States ovarian cancer 5-year survival rates by age and stage

Age (yr)	*Stage I (%)*	*Stage II (%)*	*Stage III-IV (%)*	*Unstaged (%)*	*All stages (%)*
≤45	87	66	41	56	65
45-54	84	64	24	33	47
55-64	75	56	16	30	34
65-74	79	56	11	13	28
≥75	83	59	7	16	25
ALL	82	60	17	26	38

additional 5-year survivors in the hypothetical screened population. In our model lead time does not contribute to the additional survival. Calculation of the number of screening tests required for each additional 5-year survivor assumes that tests are performed on an annual schedule; another periodic schedule may prove to be more appropriate. The female population at risk was estimated by taking 1986 United States female population figures[13] and subtracting the estimated proportion of women who underwent bilateral oophorectomy in each age group.[14]

The number of false-positive screening tests that would occur was calculated for specificities of 90% and better. Specificity is the percentage of test results that are negative among women without the disease. The number of true-positive test results that would occur in the same population was calculated for test sensitivities of 70%, 80%, and 90%. The positive predictive value of the test was calculated for each combination of sensitivity and specificity. The positive predictive value is the percentage of all positive test results that are true positive.

In a randomized controlled trial of screening the 5-year survival rate for screened cases would be a result of both decreased mortality and lead time; therefore our calculations to estimate the sample size needed for a randomized controlled trial considered total cohort mortality and not case survival rates. We present the results for a trial that would be able to detect a 50% reduction in mortality that resulted from screening with a one-sided significance level of 0.05 and power of 0.80.[15] We chose a one-sided significance level because we had no suspicion that there would be an increase in ovarian cancer mortality as a result of screening. Calculations throughout were performed for 5-year age groups wherever sufficiently detailed data were available, but for brevity results are presented in less detail.

Results

About 20,000 cases of ovarian cancer occur annually in the United States; these cases are distributed by age and stage as shown in Table I. Sixty-five percent of these cases are diagnosed at stage III or worse, and 85% occur in women older than 45 years. Of these 20,000 cases there will be about 7600 5-year survivors. The percentage of 5-year survivors by age and stage is shown in Table II. Young women have better survival both because they tend to have the diagnosis made at earlier stages and because they experience better stage-specific survival rates than do older women. The effect of universal use of a screening test with 80% sensitivity would be to increase the number of 5-year survivors (at all ages) from 7600 to 14,400. Nearly all of this benefit would occur among women with screening and diagnosis after age 45 (Fig. 1); from age 45 to age 74 the number of 5-year survivors would increase from 4700 to 9350.

Fig. 2 demonstrates the number of screening tests that would need to be performed in each 5-year age group to gain one additional survivor per year. Because cases are rare and survival is relatively good below age 45, ≥40,000 screening tests would be required to yield a single additional survivor at such young ages. From age 45 to 74 there are about 27 million women with at least one ovary who would be candidates for screening.

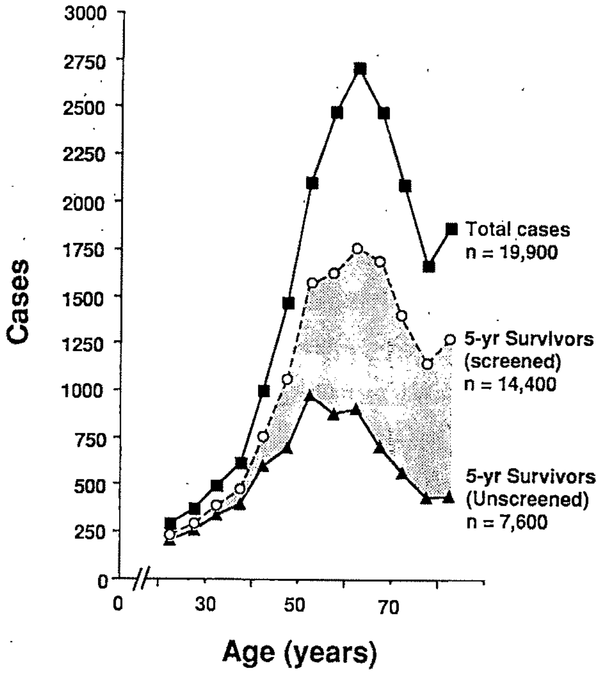

Fig. 1. Ovarian cancer cases and 5-year survivors by age. Unscreened and screened population. Screening test with universal uptake and 80% sensitivity.

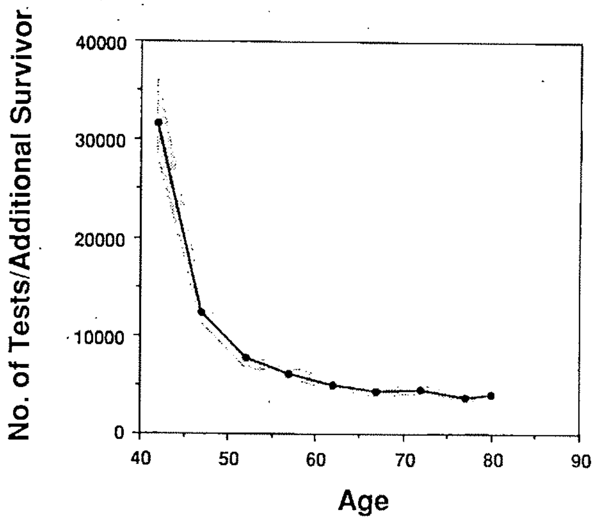

Fig. 2. Number of screening tests required to gain one additional 5-year survivor (by age). *Solid line* indicates test with 80% sensitivity; *surrounding band* includes all sensitivities from 70% *(upper bound)* to 90% *(lower bound)*.

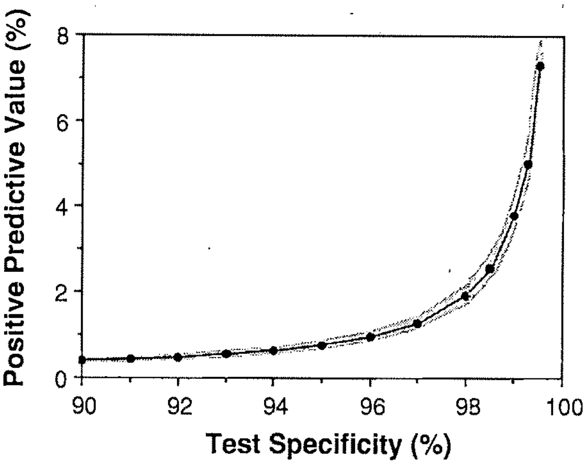

Fig. 3. Positive predictive value of screening according to test performance. *Solid line* indicates test with 80% sensitivity; *surrounding band* includes all sensitivities from 70% *(lower bound)* to 90% *(upper bound)*.

Among these women approximately 6000 tests would be required to gain an additional survivor if the test were 80% sensitive. If it were 70% sensitive, then 6850 tests would be required to gain an additional survivor. If the sensitivity of this hypothetical test increased to 90%, then about 5300 tests would be required to gain an additional survivor in this age group.

The most optimistic data on stage IA and IB disease show 5-year survival rates that approach 95%.[10] If such survival rates applied to screening detected cases (with an assumed 80% sensitivity) from ages 45 to 74, then there would be about 11,100 5-year survivors in this age group instead of the 4700 survivors expected in the unscreened population. This equals one additional survivor for every 4400 women screened.

Fig. 3 demonstrates the improvement in positive predictive value that would occur among women aged 45 to 74 as test specificity increases. For specificities of ≤98% there would be at most one woman with ovarian cancer among 50 women with a positive screening test result. In women younger than 45 years the positive predictive values are much lower than those shown in the figure because the disease is so rare.

If the hypothetical screening test has a sensitivity of 80% and if all screening-detected cases have current stage I mortality rates, then screened women aged 45 to 74 would experience about a 50% reduction in mortality from the disease. Even such a large effect of screening would be difficult to detect in a clinical study because of the rarity of this disease. The unscreened women participating in a randomized clinical trial of annual screening would have to experience at least 80 deaths from ovarian cancer during follow-up, while an equal number of screened women would have to experience fewer than 40 deaths for the improvement in the screened group to be statistically significant. A randomized trial of annual screening that includes and monitors about 100,000 women aged 45 to 74 (50,000 screened and 50,000 unscreened) for at least 6 years would be required to have the statistical power to detect this estimated 50% reduction in mortality.

Comment

Our estimates of the maximum survival benefit that might be obtained by screening for ovarian cancer were based on cancer incidence and survival data from

SEER, which covers about 10% of the country. The population covered by SEER is unlikely to differ in any important way from the country as a whole. Our calculation of the population at risk of ovarian cancer (women with ovaries) was based on survey data from upstate New York,[14] which may not be representative of the rest of the country; national data on the prevalence of oophorectomy by age would be valuable for these (and other) calculations. We believe that the number of additional 5-year survivors is a simplistic but reasonable surrogate for the number of lives that might be saved by screening, because most of the current mortality from ovarian cancer occurs within 3 years of diagnosis.[9]

In addition to reduced mortality, possible benefits of screening not considered here might include an increase in the quality of life for women with disease or a decrease in the costs of treatment. The only way to reliably measure most benefits would be by means of a randomized trial. Such a trial could also identify and quantify the adverse effects of screening, such as the number of and consequences of false-positive screening test results and the costs of screening. Randomized controlled trials that enroll fewer than 100,000 women or that monitor these women for <6 years would be unlikely to detect even a 50% reduction in mortality. Similarly, trials that evaluate a screening test that is <80% sensitive or that include women younger than 45 years will not be able to demonstrate this benefit of screening, even if such benefit exists. Ongoing observational studies[5-7] that include a few thousand women will yield important information about the screening tests evaluated, but such small studies lack the statistical power to detect an effect of screening on mortality.

We estimate that the maximum possible benefit of screening would be to save approximately 4000 to 6000 lives per year, if all 27 million American women aged 45 to 74 with ovaries are screened periodically. Screening of younger women would have a much lower yield. The maximum benefit is unlikely to be achieved because experience with Papanicolaou smears and mammography indicates that only a minority of women at risk take advantage of screening.[3, 16] In addition, our assumption that screening-detected cases would experience stage I mortality rates may be far too optimistic.[17] Thus the number of lives to be saved annually in the United States from an ovarian cancer screening program would probably be substantially fewer than 4000 to 6000.

The development and evaluation of an effective screening test for ovarian cancer will require additional research; as a result of the limited numeric benefit of screening estimated here, the priorities in such research should be the identification of subgroups of women who are at extremely high risk for this disease and a search for tests that are highly specific, as well as the further evaluation of very inexpensive tests. It is currently inappropriate to offer tests such as CA 125 and ultrasonography for the purposes of screening asymptomatic women for ovarian cancer, because no benefits of these approaches have been demonstrated. Meanwhile, it seems reasonable to advise our younger patients who are worried about ovarian cancer that they can reduce their risk of this disease by approximately 50% if they choose to use oral contraceptives[18]; this route of prevention is available to nearly all young women and could make as great a contribution to the prevention of ovarian cancer deaths as would the best possible screening program, according to our estimates.

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Prenatal behavioral risk screening by computer in a health maintenance organization–based prenatal care clinic

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Cigarette smoking, alcohol and drug abuse, and stressful life events are significant contributors to prematurity and low birth weight in the United States. Identification and treatment of pregnant women with these risk factors require obtaining complete and accurate psychosocial histories. The purpose of this study was to determine whether a computer interview developed by our staff is appropriate for assessing behavioral risk factors for adverse pregnancy outcomes and for educating pregnant women about healthy behaviors during pregnancy. This computer interview asks about pregnant patients' perceived life stressors, diet, use of cigarettes and alcohol, and abuse of drugs. The study population consisted of 201 medically insured Hispanic and non-Hispanic white women attending a health maintenance organization–based prenatal clinic. Almost all subjects rated the computer interview favorably. Medical record reviews were conducted to compare participants' reports of cigarette, alcohol, and drug use obtained from paper-and-pencil interviews with behaviors reported during the computer interview. Although self-reported rates of smoking did not differ between the two interview techniques, a much higher percentage of women reported alcohol and drug use during the computer interview. Study participants scored significantly higher on a test measuring knowledge of the effects of stress, diet, and substances of abuse on pregnancy than did a control group. Results demonstrated the potential value of computer-interactive software programs for assessing high-risk behaviors among pregnant women in this population and educating them about healthy behaviors during pregnancy. (AM J OBSTET GYNECOL 1991;165:506-14.)

Key words: Computer, screening, prenatal education, substance abuse

Smoking and drinking alcohol are prevalent behaviors among the United States population.[1] Although public awareness of the risks of cigarette smoking and alcohol and drug use during pregnancy has increased,[2] many women report that these issues are not discussed as part of their prenatal care.[3-5] Medical personnel also have difficulty obtaining accurate information from patients about alcohol use and substance abuse. An investigation by Morrow-Tlucak et al.[6] demonstrated underreporting of alcohol use during pregnancy by a significant proportion of women. Another study by Zuckerman et al.[7] revealed that of 202 pregnant women whose urine assays were positive for marijuana and 114 women whose urine assays were positive for cocaine, 16% and 24%, respectively, had denied having used these drugs.

Patient-interactive computer interviews have demonstrated value in obtaining medical histories and educating persons about behavior-related health problems.[8-10] Although there are no published studies investigating the efficacy of computer-based interviews when used in pregnant populations, this technology is easily adapted for screening and health education in a prenatal care clinic setting.

We have developed a personal computer–based software program to identify high-risk behaviors and to educate patients about healthy behaviors during pregnancy. This program: (1) assesses the behavioral risks of pregnant women, (2) provides educational information to patients in the form of personalized feedback, provided both on the video screen and on computer-generated printouts, (3) offers a resource directory of specialized health care services available to assist high-risk patients, and (4) collects anonymous prevalence data regarding reported behavioral risk factors in the population under study. Topics addressed during the interview include cigarette smoking, alcohol and drug use; optimal eating habits during pregnancy; and the importance of stress management.

We wish to report the results of a study conducted among medically insured Hispanic and non-Hispanic white women attending a health maintenance organization–based prenatal clinic. The objectives of the study were (1) to determine whether this patient-

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interactive computer interview is well accepted in this population, (2) to determine the educational value of the program, (3) to determine the validity of self-reported information on cigarette and drug use provided during the computer interview, and (4) to compare reports of substance abuse provided during the confidential computer interview with that currently being obtained through patient self-reports of high risk behaviors on a written questionnaire.

Material and methods

Description of the computer program. The Pregnancy Information Program has two important features: a flexible format, which allows programming and text changes to address specific needs of differing patient populations, and strict confidentiality protection. It is divided into eight sections: general information, stress, nutrition, cigarette smoking, alcohol use, use of other drugs of abuse, program evaluation, and printouts. The program was developed with the Knowledge Man data base management system (Micro Data Base Systems, Lafayette, Ind.) and is designed for use by persons with no computer experience or typing skills. The program text consists of multiple choice questions, written at a sixth- or seventh-grade reading level, that can be answered by typing in a number. A fiberglass overlay covers the keyboard, exposing only the number keys and the return key. The subjects can answer all questions by entering one of 10 numbers and pressing the return key. The subjects' answers are checked by the Pregnancy Information Program to determine if they are in the correct range, and they are verified by the subject. Feedback is provided by the software's simple graphics in a range of screen colors. The program collects information about the patient's eating habits, life-style practices, and life situations; explains known health effects of the behaviors under investigation; provides individualized visual and printed feedback—immediate information about how the patient's behaviors may be affecting herself and her fetus; asks the participant to rate the computer program; and generates a participant and a provider printout summarizing the assessment.

The format of this interview is similar to that of the Lifestyle Assessment for general adult medical patients developed by Allen and Skinner[11] and has features similar to health risk appraisals used in community health promotion programs. However, no risk estimates are provided in the Pregnancy Information Program. Since patients may equate risk with cause and may not understand the concept of risk, the program does not use the term *risk*. Feedback is given in simple terms, such as "studies have shown that smoking can harm the baby" or "your baby could be healthier if you stop drinking alcoholic drinks while pregnant." When statements include the concept of risk, the wording is as follows: "this may increase the chance that" or "you are more likely to have a healthy baby." The feedback information is not a fear message and does not exaggerate the health effects of the behaviors discussed.

Efforts were made, when possible, to use preexisting, validated questions for specific sections of the program. A description of each section follows:

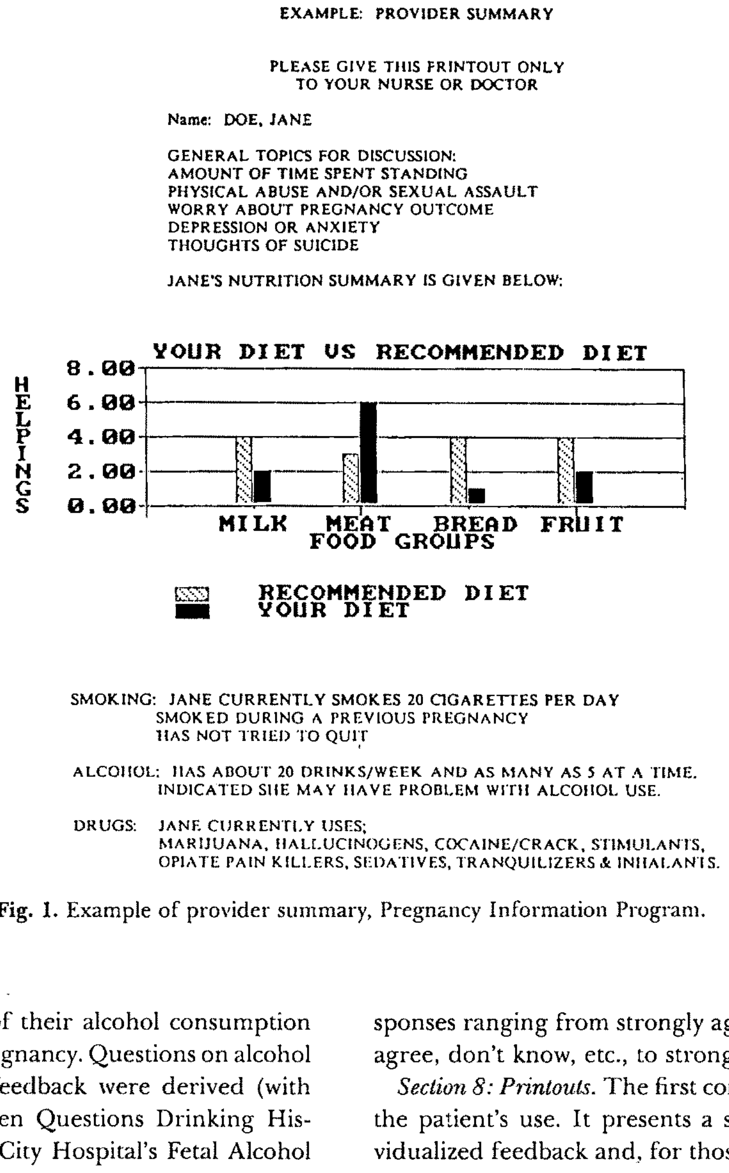
Section 1: Demographic information. This section requests the following: name, date of birth, weeks' gestation, number of previous pregnancies, number of children, years of education, ethnicity and race, number of children and adults in the household, marital status, and income.

Section 2: Stress (psychosocial risk). The computer program includes questions designed to identify women experiencing symptoms of psychologic distress. Specific questions ask whether the patient is the victim of domestic violence or is living with an alcohol or drug abuser. Questions also are asked about the patient's own perception of her level of stress, anxiety, and depression; whether she has support from family members or friends; her usual methods for dealing with stress; and whether the pregnancy was wanted.

Section 3: Nutrition. A complete assessment of the adequacy of dietary intake is not possible within the limitations of a brief computer program. The Pregnancy Information Program simply provides examples of foods belonging to each of the four basic food groups, defines a serving size, and asks the patient about her usual daily intakes of servings from each group. Feedback is consistent with current guidelines for optimal dietary intakes provided by the Women and Infant Care Guidelines.[12] Information provided to participants includes education about optimal weight gain during pregnancy and reinforcement that meals should be balanced according to the basic food groups.

Section 4: Cigarette smoking. Questions were designed to collect accurate information on this and other sensitive topics in a nonthreatening manner. Information gathered includes whether the participant smoked during previous pregnancies, number of cigarettes smoked before becoming pregnant, number of cigarettes currently smoked daily, previous attempts to quit, the participant's view of how smoking may affect her own health and the health of the fetus, and the smoking status of the baby's father. Some of the questions used (with permission) in this interview were developed by Roger Secker-Walker, MD, Professor of Medicine, University of Vermont, Burlington. Feedback and education stress the positive benefits of quitting smoking and effective methods to quit.[13] A special section written for smokers not only provides information about smoking during pregnancy but also attempts to dispel some of the myths about quitting smoking. The program then provides a short self-help method for quitting.

Section 5: Alcohol use. Patients were asked about the



EXAMPLE: PROVIDER SUMMARY

PLEASE GIVE THIS PRINTOUT ONLY
TO YOUR NURSE OR DOCTOR

Name: DOE, JANE

GENERAL TOPICS FOR DISCUSSION:
AMOUNT OF TIME SPENT STANDING
PHYSICAL ABUSE AND/OR SEXUAL ASSAULT
WORRY ABOUT PREGNANCY OUTCOME
DEPRESSION OR ANXIETY
THOUGHTS OF SUICIDE

JANE'S NUTRITION SUMMARY IS GIVEN BELOW:

YOUR DIET VS RECOMMENDED DIET
HELPINGS
8.00
6.00
4.00
2.00
0.00
MILK
MEAT
BREAD
FRUIT
FOOD GROUPS
▨ RECOMMENDED DIET
■ YOUR DIET

SMOKING: JANE CURRENTLY SMOKES 20 CIGARETTES PER DAY
SMOKED DURING A PREVIOUS PREGNANCY
HAS NOT TRIED TO QUIT

ALCOHOL: HAS ABOUT 20 DRINKS/WEEK AND AS MANY AS 5 AT A TIME.
INDICATED SHE MAY HAVE PROBLEM WITH ALCOHOL USE.

DRUGS: JANE CURRENTLY USES;
MARIJUANA, HALLUCINOGENS, COCAINE/CRACK, STIMULANTS,
OPIATE PAIN KILLERS, SEDATIVES, TRANQUILIZERS & INHALANTS.

Fig. 1. Example of provider summary, Pregnancy Information Program.

frequency and quantity of their alcohol consumption before and during this pregnancy. Questions on alcohol use and corresponding feedback were derived (with permission) from the "Ten Questions Drinking History" used at the Boston City Hospital's Fetal Alcohol Education Program, Boston.[14]

Section 6: Use of drugs of abuse. Patients were asked specific questions about their present and prior use of marijuana, amphetamine-like drugs (including cocaine), opiates, barbiturates (and other "downers"), tranquilizers, hallucinogens, and inhalants. The assessment questions were developed by Drs. Allen and Skinner for their Lifestyle Assessment[11] and were modified and adapted for the Pregnancy Information Program.

Section 7: Computer program evaluation. The computer interview also queries whether the participant liked the program; learned anything new about each of the subjects covered; was honest; would have been more or less honest if interviewed in person or by a paper-and-pencil interview; would prefer a computer, face-to-face, or paper-and-pencil interview; and whether the program took too much time to complete. All questions are answered with a seven-point rating scale, with responses ranging from strongly agree, agree, somewhat agree, don't know, etc., to strongly disagree.

Section 8: Printouts. The first computer printout is for the patient's use. It presents a summary of her individualized feedback and, for those who have identified a behavioral risk factor, provides educational material and a menu of options for obtaining information or assistance in changing unhealthy behaviors or situations. Included in this menu are options for specialized care available to the patient at the care-giving institution, lists of community resources, and lists of self-help literature. This menu can easily be custom programmed to meet the specific needs of individual offices, clinics, or institutions. The second printout is a one-page provider summary (Fig. 1). The program encourages women to share this printout with their providers.

Procedures. All patients who were within the first 20 weeks of pregnancy were eligible to participate. The study was conducted at the Lovelace Medical Center, Albuquerque, from January through December 1988. At the time of the study about 30 to 40 patients per week underwent their first prenatal examinations at the prenatal clinic. The study protocol was approved by the

Lovelace Medical Center's Institutional Review Board.

Participants were recruited by a trained project associate who explained the study to prospective subjects and asked them to provide informed consent. All participants were told that the information collected during the interview would remain strictly confidential and would not be given by the investigators to their health care providers. All women who agreed to participate received the computer program. On completion of the Pregnancy Information Program, the women were given both computer printouts. Participants had the option of giving the provider summary to their physicians. Attempts were made to enroll all eligible patients. Those who refused were asked to complete a postcard survey anonymously, containing such information as age, ethnicity, marital status, number of pregnancies, education level, and employment status, to assess possible biases introduced by patient self-selection into the study.

Urine specimens were obtained from all study subjects at the time of the computer interview, to validate their reports of nonuse of cigarettes and use or nonuse of drugs of abuse. All participants received the Wide Range Reading Achievement Test (Jastak Associates, Inc., Wilmington, Del.) before completing the computer program. Most subjects received the program before their first prenatal visit. All participants participated in the standard program for prenatal care and health education offered by Lovelace Medical Center.

Smoking status was determined by measuring cotinine levels in urine. Cotinine is a direct metabolite of nicotine and, unlike nicotine, has a relatively long half-life.[15] Cotinine can be quantified by a radioimmunoassay technique developed by Langone et al.[16] and has been shown to be a valid indicator of tobacco smoke exposure.[17] Furthermore, cotinine levels in urine and saliva have been shown to correlate well with those in plasma.[17] Urine cotinine and creatinine ratio analyses were performed at the Clinical Research Center Laboratory, University of New Mexico School of Medicine, Albuquerque. Urine cotinine measurements >49.6 ng/ml were considered presumptive evidence of active smoking.[18]

Urine samples were screened by radioimmunoassay at the University of New Mexico Toxicology Laboratory for the following classifications of drugs of abuse: opiates, cocaine and other stimulants, tetrahydrocannabinoids, benzodiazepines, and barbiturates. Positive results were verfiied at the same laboratory by gas chromatography–mass spectrometry.

To evaluate the educational component of the Pregnancy Information Program, a 33-item test was designed by Lovelace research staff to evaluate participants' knowledge of the four basic food groups, the definition of stress, the effects of cigarette smoking, and the effects of use of drugs of abuse during pregnancy.* This test was validated among patients at the Lovelace Medical Center clinic; then it was administered to 169 subjects who had received the Pregnancy Information Program from 12 to 20 weeks earlier in their pregnancies and, finally, to a comparison group consisting of 66 patients in the third trimester of pregnancy who were receiving prenatal care at Lovelace Medical Center and who had not been offered the computer program.

After each participant gave birth, a trained abstractor, who did not know the purpose of the study and had no information regarding the responses given by participants to the computer interview, reviewed each of the medical records. Chart reviews were conducted for 196 (97%) of the 201 subjects. Five medical records were missing or unavailable, and five additional records contained incomplete information. Information abstracted from the medical record included participant responses to a written questionnaire that queried the patient's use of cigarettes, alcohol, and drugs of abuse. This questionnaire was administered to participants in a group setting as part of a prenatal education class. Women completed this questionnaire 1 day to 4 weeks before they received the computer interview.

Data from the computer-assisted program were stored on the personal computer's hard drive and on floppy diskettes. Data were then transmitted by floppy diskette to a minicomputer for storage and subsequent data analysis. Pregnancy Information Program information, laboratory results, results of the reading tests, and information collected from the medical records were entered directly into a computer file. All data collected for each subject were expanded into single sequential records. Records were processed and analyzed statistically by a series of SAS (Statistical Analysis Systems Institute, Raleigh, N.C.) programs.

Results

Participation rate and characteristics of participants. Of the 229 eligible women who were asked to participate in the study, 201 (88%) agreed to participate. One woman agreed to take the program but refused to provide a urine specimen. The most commonly used reason for nonparticipation among the 28 nonparticipants was "not enough time" (54%), followed by "not interested in the study" (25%). One woman expressed a dislike of computers, and one woman felt her reading skills were too poor.

Characteristics of participants and nonparticipants were similar. The study population was almost equally divided between Hispanic (48%) and non-Hispanic (49%) white women. Ages of participants ranged from

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*Copies of this questionnaire are available from the authors on request.

Table I. Participant ratings of computer interview

	Response (%) (N = 201)						
Topic and statement	*Strongly disagree*	*Disagree*	*Somewhat disagree*	*Don't know*	*Somewhat agree*	*Agree*	*Strongly agree*
Acceptability							
I like this interview	1	<1	3	2	8	56	30
I found this interview easy to read and understand	1	1	<1	0	4	45	49
I would rather be interviewed by a person than a computer	12	33	14	14	11	12	4
I would prefer a paper-and-pencil questionnaire to a computer interview	38	41	10	5	1	4	1
Educational value							
I learned new information about:							
Stress	5	13	8	2	28	34	10
Nutrition during pregnancy	3	14	6	<1	25	38	14
Smoking during pregnancy	13	26	6	3	17	24	11
Alcohol and drug use during pregnancy	13	23	6	2	17	26	13
Perceived value as a motivator of behavior change							
I will change my life-style habits because of this interview	3	16	5	13	32	21	10
Validity/confidentiality							
I was totally honest during this computer interview	3	1	1	2	2	34	57
I would be less honest if my answers were given to my doctor and added to my medical chart	27	39	5	7	8	9	5
I will give my doctor the report of my life-style habits	1	5	<1	26	7	40	21
I was more honest in this interview than I would be in a personal or paper-and-pencil interview	10	25	5	8	9	24	19

17 to 42, with a mean of 28 years. The mean number of years of school completed was 13, and all but one of the participants read at a minimum eighth grade reading level. Approximately 74% were married; the current pregnancy was the subject's first for 26% of the participants. The majority of the subjects were employed full-time (59%) or part-time (13%). Almost all of the participants either were members of the Lovelace-affiliated health maintenance organization (81%) or had other health insurance (16%).

Participant ratings of the computer interview. Almost all (94%) of the participants liked the computer interview (Table I). Only 6% of the patients agreed with a statement indicating they would prefer a written questionnaire to the computer interview (Table I). Computer interviews conducted among the 201 subjects in the Lovelace population averaged 30 minutes to complete; 11% of the participants thought it took too much time to complete.

Health education value of the computer program. Most of the study participants (76%) reported during the evaluation section of the computer interview that they "somewhat agreed, agreed, or strongly agreed" with a statement indicating they learned new information about at least one of the topics included in the interview. The subject about which the highest percentage of women reported learning new information was nutrition (77%) (Table. I). Using general linear model regression, younger age, fewer years of education, lower income, and Hispanic ethnic group each were found to be positively associated with responses indicating the participants learned new information about the risk factors discussed in the Pregnancy Information Program.

The mean score on the test measuring knowledge of behavioral risk factors was 83% correct responses for study participants, compared with 79% for the controls ($p = 0.01$). Participants, however, were older and had higher income levels than controls, requiring use of a statistical model to further assess whether the relationship (between higher scores and having taken the computer program) would persist after controlling for the effects of these two factors. A general linear models procedure was used, in which age, exposure to the computer program, income, ethnicity, and years of education were independent variables, and score on the test was the dependent variable. The overall R^2 of the model was 0.40, and the p value for having taken the computer interview was 0.04, indicating a positive relationship between scores on the test and having received the computer interview.

Validity of the computer interview

Self-reported honesty. Ninety-three percent of the Lovelace participants agreed with the statement that they were "totally honest" during the computer interview; however, 5% disagreed with this statement and 2% responded "don't know" (Table I); 52% agreed with the statement that they were more honest to the computer than they would have been in a personal or paper-and-pencil interview. In response to the statement, "I will give my doctor the report of my life-style habits," 52 women (26%) reported "don't know" and 143 women (68%) agreed (Table I). More than 20% reported they would be less honest if their answers were given to their doctors and added to their medical charts.

With the answers provided during the evaluation section of the computer questionnaire, it was possible to determine factors that were associated with reported honesty and willingness to share information with the participants' providers. Statistical comparisons were drawn between participants' demographic characteristics and their self-reported behaviors, to further elucidate the relationship between reported risk factors, and statements about reported honesty and feelings about the confidentiality of the computer interview (Table I). With general linear model regression, current use of alcohol and drugs was found to be associated with a higher degree of agreement with the statement, "I would be less honest if my answers were given to my doctor and added to my medical chart" ($p = 0.001$ for drug users and $p = 0.014$ for alcohol users). Those who used drugs also were more likely to report higher levels of agreement with the statement, "I was more honest in this interview than I would be in a personal or paper-and-pencil interview" ($p = 0.004$), and to have lower levels of agreement with the statement, "I will give my doctor the report of my life-style habits" ($p = 0.0001$).

Urine testing. Of the 201 women enrolled in the study, 17% stated they were current smokers. A random sample of 71 urine specimens was analyzed for cotinine levels from among the 167 women who stated that they were currently nonsmokers. Results revealed that 7% of these women had urine cotinine levels indicative of active smoking (Table II).

Data regarding use of drugs of abuse (Table III) indicated that few of the participants used illicit drugs. Eleven women admitted to current marijuana use. One additional woman reported current use of cocaine. Of 67 women who did not report current drug use, and whose urine specimens were randomly selected and assayed for drugs of abuse, 2 (3%) were positive.

Table II. Urine cotinine concentrations among participants

Reported smoking status	*Elevated cotinine**	*Discordance*	*95% Confidence interval*
Never smoked ($n = 39$)	1	5/71 (7.0%)	2.3%–15.7%
Quit smoking ($n = 32$)	4		

*Urine cotinine level >49.6 ng/mg of creatinine.

Table III. Results of urine assays for drugs of abuse among participants

Reported drug use	*Positive test (substance)*	*Discordance*	*95% Confidence interval*
No ($n = 67$)	2*	2/67 (3.0%)	0.4%-10.4%
Yes ($n = 3$)	1*†; 2, Negative‡		

*Tetrahydrocannabinoids.

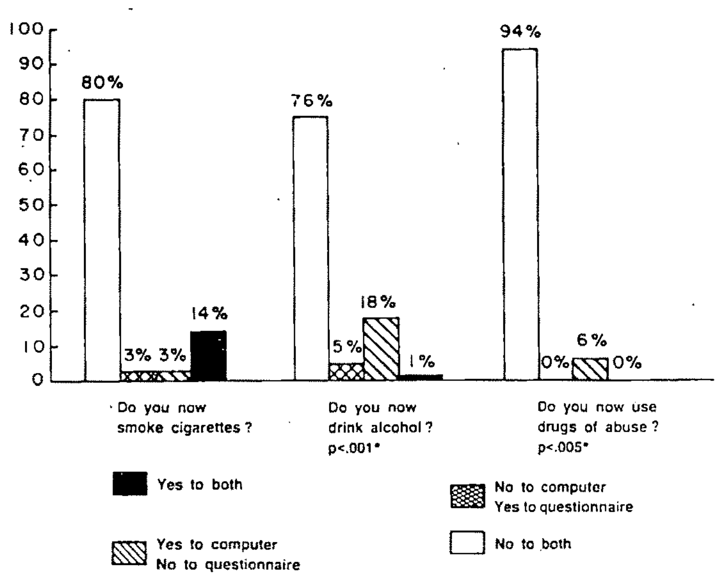
†Reported last use 7 days ago.

‡Reported last use of marijuana 7 days ago and >4 weeks ago. Both reported frequency of use as less than once per week.

Comparisons between reports to the computer and information in the medical record. Comparisons between behaviors reported by patients to the Pregnancy Information Program and patients' responses to a written questionnaire placed in the medical chart revealed that the computer interview obtained results similar to those of the written questionnaire for ascertaining smoking status (Fig. 2). However, a statistically higher percentage of subjects reported alcohol and drug use to the computer than they reported in the written questionnaire (Fig. 2). Of the 47 discrepancies noted between alcohol use recorded in the medical chart and behaviors reported in the computer interview, in all but five instances the discrepancy was accounted for by a negative patient report in the written questionnaire and a positive report of alcohol use in the computer interview. There was no reported use of substances of abuse in the medical record, compared with 11 reports of marijuana use during pregnancy and one report of cocaine use provided during the computer questionnaire.

Comment

The results of this study demonstrate that computer interviews can be a useful adjunct to prenatal care among well-educated, medically insured, white, Hispanic and non-Hispanic patients. The Pregnancy Information Program format was well received by study participants. Ninety-four percent of the women enrolled in the pilot study indicated that they liked the



*McNemar's Paired Sample Test of Nominal Scale Data

Fig. 2. Comparisons between reports to computer and responses to written questionnaire.

computer interview, and almost 95% of the participants preferred the computer interview to a written questionnaire. Of the 201 women in the study, only about one in four (27%), "somewhat agreed" to "strongly agreed" with the statement that they would rather be interviewed by a person than by a computer. A potential problem reported in the literature—that some individuals are uncomfortable with or afraid of computer interviews[19]—was not a problem in this study. None of the participants was intimidated by the computer interview and only one of the nonparticipants indicated that her lack of interest in participating was due to a dislike of computers. The high participation rate in this study indicated a strong interest among women in this population to experience new health education approaches. Therefore the health maintenance organization setting may be one that is optimal for use of patient-interactive computer programs.

Results of this study also support the premise that computers can be useful for patient education. More than 75% of women who completed the Pregnancy Information Program reported agreement with the statement that they learned new information from the program. Scores on a test measuring knowledge of the risk factors discussed in the program were higher among study participants than among controls who were not offered the program. These results support the work of Paperny and Starn,[20] who have developed color graphics computer games that provide educational information for adolescents about unhealthy behaviors. In an evaluation of two of the programs, *Babygame* and *Romance*, users of the program became more aware of factors such as the cost of giving birth to and raising a child and they became more knowledgeable about the safety and efficacy of contraceptives.[20] The current prenatal program at Lovelace Medical Center contains a patient education component. Most of the women in the control group scored well on the test, indicating awareness of the possible consequences of high-risk behaviors during pregnancy. Therefore it was not possible to evaluate the impact of the computer-assisted program as a singular entity but rather as an adjunct to the specific program of prenatal care offered by the study institution. In spite of this and the relative simplicity of the Pregnancy Information Program, most of the women stated they learned new information. Many participants also indicated that the program would motivate them to make behavioral changes; 68% stated they would change their behavior after the computer interview. However, whether changes actually occurred must be determined in further studies.

The third objective of the study was to determine the validity of the Pregnancy Information Program in obtaining accurate behavioral risk information. Rates of participants' self-reported honesty and results of the urine assays conducted for this study support the conclusion that the information provided by participants was valid. The concordance rates—between self-reports of smoking and drug use and results of urine assays—were 93% and 97%, respectively. However, these screens will detect recent users only. Most of the urine assays for drugs of abuse are not sensitive enough to detect drugs taken >3 or 4 days before sampling. Exceptions include marijuana (detectable for as long as 30 days for a heavy marijuana user, 7 to 10 days for a moderate user, and 3 to 5 days for a light user), phenobarbital (detectable for 2 to 3 weeks), methaqualone (detectable for 2 weeks), and phencyclidine (detectable for 8 days).[21] In addition, urine tests are not 100% sensitive and specific. They were performed on a relatively small cohort and were obtained at one time point. Therefore some participants may have been misclassified as being truthful (false negative) or untruthful (false positive). Subjects were informed that the urine specimens provided may be tested for cotinine and drugs of abuse. Some of the participants could have been more honest in reporting smoking and drug use because they did not want their test results to belie their answers to these questions. Finally, testing did not include a screen for recent alcohol use.

Previous studies have found that computers may obtain more accurate information than that obtained in a face-to-face interview.[22, 23] Skinner and Allen[24] found that people were equally honest with computer-based interviews as with face-to-face or paper-and-pencil questionnaires in reporting use of alcohol, tobacco, caffeine, and drugs.

The fourth objective of the study was to compare responses to the Pregnancy Information Program with self-reported use of cigarettes, alcohol, and drugs of abuse obtained by a written questionnaire, completed as part of routine care in this prenatal clinic. Study participants did report significantly more drug and alcohol use during the computer interview; there was no difference in the rate of reported smoking. In the majority of instances, the discrepancy occurred because the participant denied the behavior during the written questionnaire and reported the behavior in the computer interview. However, there were a small number of exceptions, where a subject reported the behavior in the written questionnaire and denied it during the computer interview.

There are several possible explanations for the discrepancies between reports obtained from the two questionnaire modalities. The most likely explanation is that the women were more honest in reporting their behaviors during the computer interview. Conceivably women who were not smoking, drinking alcohol, or using drugs at the time they attended the prental class initiated these behaviors in the period between taking the written questionnaire and the computer interview. However, this seems unlikely. Available evidence indicates that a high percentage of women abstain from or reduce smoking and drinking alcohol soon after discovering they are pregnant.[25, 26]

Some of the participants may have reduced their high-risk behaviors as a result of the information provided during the educational class before receiving the computer program. This could account for the small number of persons who reported the behavior initially during the written questionnaire and failed to report the behavior during the computer interview. Some participants also may have misread or misunderstood the questions in either questionnaire, thus accounting for discrepancies.

The computer interview was offered in a setting of anonymity. Information provided during the computer interview was not made available to the patients' physicians; however, included in the on-screen information for the participants was a statement urging them to give the computer printout entitled "Provider Summary" to their physicians. An informal survey of providers conducted after this study was completed revealed that few printouts were actually received by providers. This is in contradistinction to the high percentage of women who stated they would give the report to their providers.

In a recent study of pregnant women[6] the authors found that those with a history of alcohol-related problems, determined by an elevated score on the Michigan Alcoholism Screening Test, were most likely to underreport alcohol consumption. In this study a large number of participants did not wish to disclose the results of their interviews to their physicians. The persons who were least willing to share their life-style information with their physicians were current drug and alcohol users. This finding supports the conclusion that the observed increased reporting of alcohol and drug use during the computer interview was attributable to its confidential nature.

Routine use of institution-specific computer software programs, such as described here, could provide personalized health risk information to all patients, including those women who do not report their behavior accurately to their health care providers. The software could be programmed to direct patients to an appropriate referral source and could optimize the use of existing services within the institution. The providers administering the interview would obtain prevalence data, which could be used to guide medical staff in making informed decisions about resource allocations. In addition, anonymous patient-interactive computer programs have demonstrated value in increasing dialogue between patients and their physicians in a general medical practice. Skinner et al.[8] found that patients were more likely to discuss life-style issues with their physicians if they received a computerized life-style assessment before seeing their physicians. If the Pregnancy Information Program were used as part of routine care, the provider summaries, when patients chose to share them, could serve as a focus for discussions regarding high risk for adverse pregnancy outcomes.

This study demonstrated that a confidential, computerized, patient-interactive assessment was valid in this patient population. Its use in prenatal clinics could enhance prenatal education programs and provide information useful for institutional planning and resource allocation.

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Short-term variation in abnormal antenatal fetal heart rate records

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In a retrospective study the relation of reduced fetal heart rate variation to fetal acidemia was analyzed with a computerized system for numeric analysis. Between 1983 and 1987, 78 pregnancies were identified in which at least one record of the fetal heart rate had very low long-term variation. The outcome was analyzed to determine the numeric criteria of fetal heart rate variation that most efficiently detect a fetus that will die (preterminal) or is dying (terminal). Because fetal compromise was found on occasion to be associated with a slow sinusoidal fetal heart rate rhythm that increased measures of long-term variation. It was necessary to define a new index of short-term fetal heart rate variation (the 1/16 minute epoch-epoch variation). This was closely related to long-term variation ($r = 0.9$) but provided better detection of preterminal records as judged by metabolic acidemia at delivery or intrauterine death. (AM J OBSTET GYNECOL 1991;165:515-23.)

Key words: Fetal heart rate variation, terminal fetal heart rate records, computer analysis, intrauterine death

Evaluation of the fetal heart rate (FHR) is a clinical test of well-being, that is based on empiric observations, of which many aspects are poorly understood. Grossly abnormal patterns have been identified by visual analysis but with large interobserver and intraobserver variation.[1-4] The degree of such variation is so great as to cast serious doubts on the accuracy of current guidelines. To overcome the problems of subjective analysis, we have developed a system for numeric analysis of the human FHR, with a small on-line computer.

The analysis depends on accurate fitting of the baseline and on simple techniques for measuring heart rate variation, accelerations, and decelerations. By quantifying signal loss, the quality of different recording systems can be assessed[5]; the introduction of a wide-range ultrasonographic transducer, combined with autocorrelation, improved record quality.[6]

Originally we started from the observations of Visser and Huisjes[7] that identified decelerative FHR traces as a cause for concern. However, the feature that was outstandingly associated with acidemia, on delivery by cesarean section in the absence of labor, proved to be a decline in FHR variation; hence the focus of this article. Its shortest component (beat-to-beat variation) is too small to be measured accurately by the present generation of FHR monitors and cannot be reliably estimated by external ultrasonographic monitoring.[8, 9] Measurement of longer-term FHR variation provided accurate information on the rest and activity cycles of the normal fetus[5, 10, 11] and evidence of compromise associated with chronic hypoxemia. An index of long-term variation, the mean minute range, was devised, in which the effects of decelerations are excluded to identify records that are otherwise flat. As gestational age advances, the mean minute range increases a little, but much less than the incidence of accelerations; at term its normal value is about 50 milliseconds corresponding to about 17 beats/min.[6] Abrupt changes in the magnitude of the mean minute range identify episodes of low or high FHR variation, coincident with changes in fetal rest and activity from 28 weeks' gestation onward. We have shown that a 5-minute episode of active fetal sleep, identified in this way, is a better index of health than reactivity defined in terms of FHR accelerations.[12]

FHR variation was much reduced in association with intrauterine growth retardation without acidemia.[13] This resulted from attenuation of the amplitude of variation rather than from prolongation of episodes of low variation.[14] The mean minute range was reduced from a normal value of 44 ± 1.5 milliseconds at 32 weeks to 20.6 ± 1.2 milliseconds, and this was associated with a decrease in fetal Pa_{O_2} measured at cesarean section before labor and with hypoglycemia, hyperalaninemia, and a rise in amniotic fluid erythropoietin as evidence of chronic hypoxemia.[15] Hypoxemia in these circumstances has been confirmed by cordocentesis.[16]

In these studies the attributes of the FHR of the

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chronically compromised fetus were defined, but the absence of acidemia at birth confirmed that the fetal state was not terminal. The concept of preterminal and terminal FHR records[7] has helped to identify the fetus that requires immediate delivery. In modern practice the qualities of the terminal trace cannot be studied formally because, once recognized visually, immediate intervention is required. However, to examine the limits of intrauterine survival in relation to FHR variation, we reviewed all cases encountered over 4 years (1983 to 1987) in which at least one record had an abnormally low mean minute range (<20 milliseconds), much less than the lowest with normal outcome.[17] We also identified a small number of fetuses with a terminal heart rate pattern who could not be delivered because of immaturity and who died in utero. In one a slow sinusoidal rhythm was superimposed on an otherwise flat FHR, in such a way as to yield a high mean minute range. We therefore explored the relation between short-term and long-term measures of FHR variation in preterminal and other records and derived a new index of short-term variation to improve diagnostic discrimination.

Methods

FHR records were taken with Hewlett-Packard model 8040 fetal monitors. The data were captured and analyzed by a microcomputer on line. From 1983 to 1985 the records were made with Nascom microcomputers.[5, 18] From 1986 these were progressively replaced with Apricot XEN microcomputers, which accessed the RS232 port of the HP8040. The archive of Nascom disks was transferred to the new system and reanalyzed. The date and time (to the nearest minute) of the start of the record was entered manually (Nascom) or automatically (Apricot XEN).

The analysis proceeded as follows. The autocorrelation function, generated by the fetal monitor as a measure to discriminate between background noise and valid signal, was sampled. Accuracy was further checked by an error algorithm.[19] Valid pulse intervals were averaged over epochs of 3.75 seconds (1/16 minute) and a baseline was fitted.[20] Signal loss was calculated as the proportion of epochs for which no valid pulse interval was available. The fetal monitor measures pulse intervals, not heart rate; therefore calculations of FHR variation were reported in milliseconds.

Large changes in heart rate from the baseline were identified as accelerations (>10 beats/min and 15 seconds) or decelerations (>10 beats/min and ≥1 minute, >20 beats/minute and ≥0.5 minute, or >25 beats/min and ≥0.25 minute). The area of decelerations was calculated as the number of lost beats,[21] and large decelerations were defined as having an area >20 lost beats. The temporal association of decelerations with uterine contractions (where present) and the lag time between peak contraction and FHR trough were recorded. In each minute the computer acquired 16 measurements of fetal pulse interval; each was averaged over a period of 3.75 seconds. The difference between minimum and maximum was calculated as the minute range. For a record of 25 minutes' duration, for example, there were 25 such values, whose average (the mean minute range) was calculated as an index of long-term FHR variation. Several adjustments were made. First, minutes in which a deceleration (defined above) occurred were excluded, so that decelerative variation did not amplify this index. In the same way minutes were excluded in which the FHR was wholly below baseline. Second, if the minute under consideration formed part of a prolonged acceleration, then the range was calculated from the baseline. These adjustments ensured that long-term variation (the mean minute range) was biased toward recording accelerative FHR variation, the main characteristic of the normal record.[22] There was a close correlation ($r = 0.97$) with the root mean square value of FHR variation.[6] Short-term FHR variation was calculated as the average of successive epochal (1/16-minute) differences, decelerations being excluded as for the calculation of long-term variation.

Certain decelerations and accelerations, with abrupt changes in FHR (>35 beats/min followed by an equally abrupt change in the opposite direction) were identified and excluded as unreliable evidence.[23] Decelerations and accelerations with high signal loss (>50%) also were eliminated as unreliable.

Episodes of high or low FHR variation were identified when in 5 of 6 consecutive minutes the mean minute range of pulse intervals was >32 or <30 milliseconds, respectively.[6, 24] If long-term variation was <22 milliseconds and there was no episode of high variation, the record was reanalyzed, with extra constraints on the construction of the baseline, to enable shallow decelerations to be identified (>5 beats/min and 0.5 minute) and their temporal relation to uterine contractions to be established. The choice of these criteria has been explained elsewhere,[5, 9, 10] and the application to fetuses of different ages has been checked by us[6, 11] and by joint studies with colleagues in other centers in Great Britain, The Netherlands, Canada, Italy, and the United States (unpublished observations). Basal heart rate was measured as the mean value during episodes of low FHR variation or, if no such episode was available, it was measured as the modal or peak value of pulse intervals (as previously described), calculated from the frequency distribution.[20] In this data set all records contained episodes of low FHR variation.

During the record the mother signaled all fetal movements with a hand-held button. Each epoch (3.75 seconds) containing one or more perceived movements was identified. The duration of the FHR trace was determined by the on-line analysis. If an episode of high variation was identified, with no large deceleration and at least one fetal movement or three accelerations, a message was displayed advising that recording may be stopped. Otherwise it lasted 60 minutes. The average record length in ordinary use was about 15 minutes. At the end of each record all FHR variables were printed, and if there were five or more records available on that patient, a synoptic display was presented in which the significance of the trend in short-term variation with time was calculated (e.g., Fig. 3). The computer program used was the same as that previously described,[6,15] with the addition of algorithms to exclude unreliable data[23] and to measure short-term FHR variation.

Clinical selection. We analyzed 7396 FHR records obtained from 2582 women from June 1983 through 1987, during which there were 23,010 births to 22,742 women in the John Radcliffe Hospital. The patients were monitored because they were identified as at high risk from medical disorders such as hypertension, diabetes, or renal disease; because of a poor obstetric history; or because of current obstetric problems, mainly proteinuric preeclampsia, or because the fetus was small for gestational age. These were cases of preterm placental vascular insufficiency, often associated with preeclampsia, in which closely judged decisions were needed to avoid premature delivery.

In 78 women the mean minute range was <20 milliseconds on at least one occasion. These women had a total of 961 antenatal FHR traces. The first record with a mean minute range (long-term variation) <20 milliseconds was defined as the index trace. The reasons for a subsequent decision to expedite delivery, by induction of labor or cesarean section, were classified before delivery as maternal (n = 9), maternal and fetal (n = 4), fetal (n = 39), or prophylactic (n = 16). In the last group neither maternal nor fetal safety was judged to be at immediate risk, that is, during the next 24 hours. Five women went into labor spontaneously. There were four intrauterine deaths and one other, very immature fetus died because the pregnancy had to be terminated urgently for reasons of maternal health at 27 weeks. The decision to deliver took into account the FHR trace, gestational age, and ultrasonographic estimate of fetal weight, a scan of fetal movements and amniotic fluid volume, and maternal condition. The principal guide to clinical management of the fetus has been the measurement of FHR variation.

Of the 57 women with proteinuric hypertension (Table I), 42 were treated with antihypertensive agents (38 with α-methyldopa, six of whom also received nifedipine), two received diazepam, and two were given a β-adrenergic-blocking agent.

Table I. Data on mothers and fetuses at time of index trace, on delivery, and after birth (mean ± SD)

Mother	
No.	78
Nulliparous	43 (55%)
Proteinuric preeclampsia	57 (73%)
Small-for-gestational-age	15 (19%)
Diabetes	1 (1%)
Maternal age (yr)	28.1 ± 7.9
FHR record (index trace)	
Gestational age (days)	221.5 ± 34.2
FHR variation (msec)	
Long-term	16.7 ± 2.7
Short-term	3.0 ± 0.6
Delivery	
Gestational age (days)	230 ± 34.3
Intrauterine deaths	5
Total live-born	
Vaginal delivery	16 (4 acidemic)
Cesarean section	57 (4 acidemic)
Umbilical artery blood pH	7.22 ± 0.02
Neonatal outcome	
Live-born	73
Neonatal deaths	4
Hyaline membrane disease	30 (41%)
Hypoglycemia	26 (36%)
Patent ductus arteriosus	18 (25%)
Mortality	9 (11%)

Acidemia on delivery was defined as an umbilical arterial pH <7.12 and a base deficit >12 mmol/L. Umbilical arterial and venous blood was sampled; measurements were made at 37° C (ABL2 Radiometer, Copenhagen). Neonates <10th percentile of birth weight for gestational age and gender[25] were defined as being small for gestational age. Hypoglycemia was defined as a blood glucose level <1.11 mmol/L for an infant <2.5 kg, otherwise as <1.66 mmol/L. Hyaline membrane disease was defined as an immature infant with increased breathing, an expiratory grunt, rib recession on inspiration, cyanosis, and a chest x-ray film with diffuse granularity and an air bronchogram.

This study is retrospective and describes the evolution of methods that were not anticipated but have evolved as clinical and analytic experience has grown. The ultimate hypothesis was that a reduced FHR variation characterized the hypoxemic and acidemic fetus.[7,13,15] Hence statistical tests were used to confirm the anticipated relation between FHR variation and fetal arterial pH or base deficit. The Student t test was used to compare mean values, and the significance of trends in FHR variation with time was calculated from the slope of the regression line.[26] Nonparametric measures (such as the Mann-Whitney U test) also were used where there was evidence of nongaussian distribution.

Results

Clinical data and outcome. The clinical details are shown in Table I. The mean gestational age at which the index FHR trace was taken was just under 32 weeks,

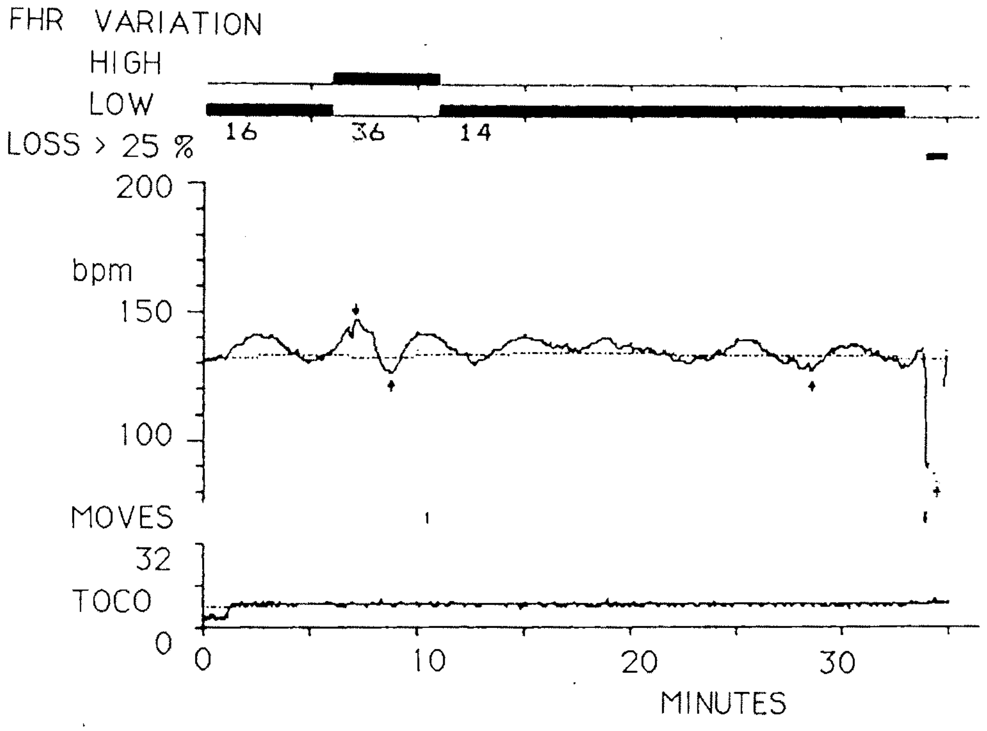

Fig. 1. Record at 28 weeks showing slow sinusoidal oscillation imposed on otherwise flat trace. Long-term variation was 18.3 milliseconds; short-term, only 1.6 milliseconds.

Table II. Incidence of acidemia* at delivery by gestational age (median values and ranges)

	Gestational age at index trace		
	<28 wk	*28 wk–31 wk 6 days*	*>32 wk*
No.	17	19	42
Days to delivery	6 (1-74)	2 (0-20)	3 (0-37)
Acidemia at delivery*	2	1	5
Intrauterine deaths	4†	1	0
Neonatal deaths	1	3	0

*pH <7.12 and base deficit >12 mmol/L, four of which were delivered after labor (three at >32 weeks).

†There was one termination of pregnancy for reasons of maternal health at 27 weeks. Two infants had Down's syndrome; one died neonatally. There were no other congenital abnormalities.

but the range was from 24 weeks 1 day to 37 weeks 6 days. One pregnancy was terminated for maternal reasons at 27 weeks. The four intrauterine deaths were predicted, but the fetuses were not delivered because of extreme prematurity.

The total of intrauterine and neonatal mortality was high (11.5%), as would be expected in this high-risk group. More than half of the deaths occurred in those in whom the index trace was taken at <28 weeks, and there were none when it was taken after 32 weeks (Table II). Table II also shows the incidence of acidemia (as defined) at delivery, analyzed by the gestational age at the index traces. Acidemia was associated with labor (four cases) or with a single FHR trace, after which delivery by cesarean section was done at once (two cases, short-term variation very low, 2.1 milliseconds in both instances), or with an exceptionally rapid fall in FHR variation (two cases). All stillbirths were anticipated as terminal FHR traces. Twenty-seven fetuses were <30 weeks' gestation when the index trace was taken; nevertheless, pregnancy was safely prolonged in 17 (63%) by an average of 17 days.

Short- and long-term FHR variation. In one patient an FHR record was encountered with a single slow sinusoidal oscillation imposed on an otherwise flat trace (Fig. 1). The amplitude of the oscillation was so large that an episode of high variation (usually a reliable indicator of normality) was identified at 6 to 11 minutes (amplitude, 36 milliseconds), and there were two episodes of low variation (16 and 14 milliseconds). The ominous significance of the record was appreciated at the time, but it was decided not to deliver the fetus because it was small (<500 gm) and immature; it died in utero during the next 24 hours. This trace demonstrated that an episode of high long-term FHR variation could occur in an otherwise terminal trace, but the variation was of a distinctive type not seen in normal records. This stimulated a reconsideration of long-term

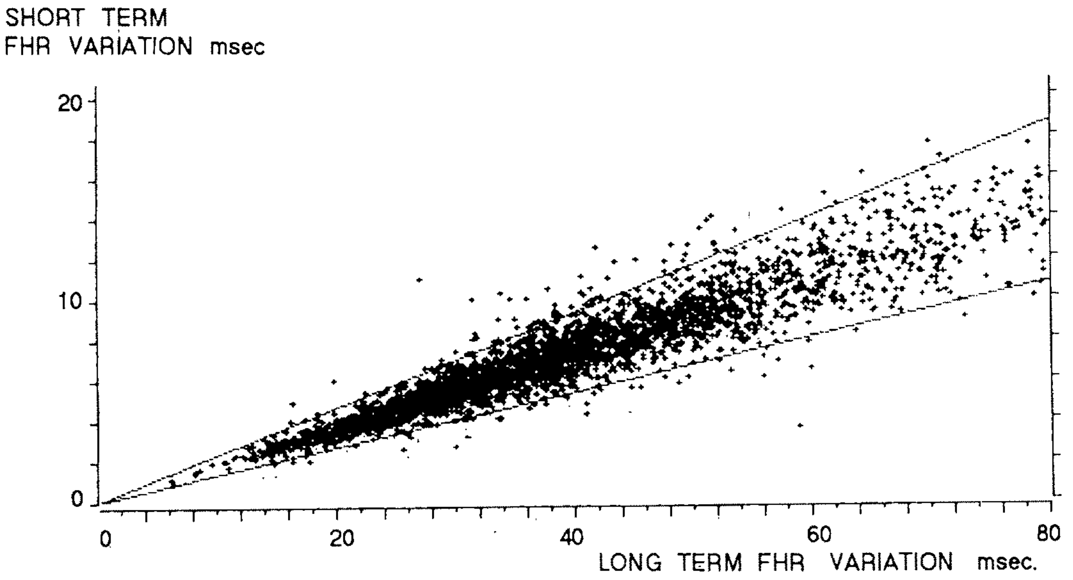

Fig. 2. Relation between short- and long-term variation in 3074 FHR traces, including 961 of this study. Mean ratio of short- to long-term variation was 0.18; *lines* are ±2 SD about mean.

variation as the only measure of FHR variation, so that the identification of terminal traces could be made more precise.

A new statistic of short-term variation was calculated as the average of the differences in mean pulse intervals in consecutuve epochs lasting 3.75 seconds (epoch-to-epoch variation). The ratio of short- to long-term FHR variation was analyzed in 2113 consecutive normal clinical records (short-term variation, 7.8 ± 3.01 milliseconds, mean ± SD) and in the 961 records of this study (short-term variation, 4.8 ± 1.85 milliseconds, mean ± SD). Because the distribution of ratios was similar, the results were combined. Fig. 2. shows this relationship, the lines being ±2 SD from the mean. The correlation coefficient was 0.90. The ratio (mean ± SD) of short-term to long-term variation was 0.183 ± 0.024; in contrast, the values for short- and long-term variation for the case illustrated in Fig. 1 were 1.6 and 18.2 milliseconds, respectively, with a ratio of 0.088, >2 SD below the mean.

We then sought how often such single sinusoidal rhythms of large amplitude occurred. There were seven records with short-term variation >2 SD below the mean value predicted from long-term variation, and with long term variation <30 milliseconds, five had simple (that is, single) sinusoidal patterns of large amplitude. The incidence of such slow sinusoidal traces was 1.6 per 1000 records (in the whole population, 7396 records) but one in nine (5/43) terminally in fetuses that were delivered by cesarean section for fetal (or fetal and maternal) reasons or died in utero. Of the three delivered alive, two were severely acidemic (pH 6.9 to 7.01; base deficit 14 mmol/L). Thus calculating the ratio of short- to long-term variation has identified rare FHR traces with slow single sinusoidal oscillations superimposed on low short-term variation. Short- and long-term variation was calculated for all records described in this report.

The next step was to analyze the relationship between the new index of short-term variation and umbilical arterial blood acid-base status at delivery and to determine the values characteristic of a terminal or near-terminal trace. For the purpose a subset of the 78 cases was selected that had at least one trace with short-term variation <3 milliseconds. This limit was chosen from a knowledge of the distribution of short-term variation in normal traces, as well as of the 961 study records, and corresponds to a mean minute range of 16.4 milliseconds (Fig. 2). There were 44 such cases (40 live births; Table III). When short-term variation decreased below 2.6 milliseconds, umbilical arterial pH was decreased and base deficit was increased, suggesting that the fetus was becoming acidemic and the record might be preterminal. This was consistent with observations on the four intrauterine deaths, for which the short-term variation was 0.9 to 2.5 milliseconds in the last trace within 24 hours of death.

The possible influence of drugs on basal FHR, FHR variation, and outcome was examined. The mothers of the four fetuses that died in utero had not received drug treatment. Nor had six of the eight found to be acidemic (pH <7.12 and base deficit >12 mmol/L) on delivery; the other two had received α-methyldopa only. In 37 patients treated with α-methyldopa and 28 untreated patients, there were no statistically significant differences between the two groups in basal FHR, FHR variation, or arterial base deficit on delivery.

Trends in short-term FHR variation before deliv-

Table III. Relationship between short-term FHR variation in last trace before delivery and umbilical arterial blood gas values

	Short-term FHR variation		
	>3 msec	*2.6-3 msec*	*<2.6 msec*
No. of live births	18	11	11
Gestational age at delivery (wk)	29.1 ± 0.8	31.8 ± 0.8*	32.3 ± 1.0
Birth weight percentile			
<10th	14	10	7
<3rd	8 (44%)	3 (27%)	3 (27%)
Umbilical arterial			
pH	7.25 ± 0.017	7.22 ± 0.027†	7.04 ± 0.07*
Base deficit (mmol/L)	6.3 ± 2.3	5.8 ± 1.3	11.9 ± 1.5*
No. with acidemia (pH <7.12 and base deficit >12 mmol/L)	0	1	3

Values are mean ± SE.

*Difference from values at >3 milliseconds of short-term FHR variation, $p < 0.01$.

†Difference from values at >3 milliseconds of short-term FHR variation, $p < 0.05$.

ery. In 44 (56%) of the 78 patients there were seven or more records on file, which could be used to analyze the trend with time in short-term FHR variation. Twenty-eight of the 44 pregnancies were terminated for fetal (or fetal and maternal) reasons, 10 prophylactically to avoid further risk (all but one at 36 to 37 weeks' gestation) and five for maternal reasons only; there was one intrauterine death. In nine cases the short-term variation fell significantly ($p < 0.05$) during the last 2 weeks before delivery. In four pregnancies it increased to a significant extent, and in the remaining 31 there was no significant trend with time. Fig. 3, *A*, shows a rapid fall in short-term variation (−0.58 millisecond per day). Fig. 3, *B*, shows a slower fall over a 2-week period. The most rapid fall (by −1.01 millisecond per day down to 2.2 milliseconds terminally) was observed in seven records taken over 4 days in a fetus acidemic on delivery by cesarean section at 28 weeks. Fig. 3, *C*, illustrates the converse, a progressive improvement, shown by a significant rise in short-term FHR variation (+0.20 millisecond per day; $p < 0.05$) over 9 days with more fetal movements; the pregnancy was terminated by cesarean section at 28 weeks for maternal reasons (symptomatic proteinuric preeclampsia). The proportions delivered for fetal reasons were 89%, 55%, and 0% associated with falling, unchanged, or rising trends in short-term FHR variation. The umbilical arterial blood pH of those delivered after a fall in short-term FHR variation was significantly lower (7.20 ± 0.019) than that of the rest (7.27 ± 0.013, $p < 0.01$) Fig. 3 also illustrates the relatively small changes in basal heart rate with time in such circumstances.

In 13 of these 44 patients there was an abrupt terminal reduction in fetal movements or long-term FHR variation (usually both), even when the trend over the last few days had not been remarkable in either direction.

Seven fetuses were delivered for fetal reasons at once (mean short-term variation, 2.5 ± 0.20 milliseconds) at 29 weeks to 33 weeks 6 days as a result of only one or two FHR records. The FHR traces of three of these fetuses had no decelerations, suggesting that the presence of decelerations may be an unreliable guide to a preterminal or terminal fetal state. The umbilical arterial pH (mean ± SE) on delivery (in all seven) was 7.09 ± 0.06). Two of these fetuses were asphyxiated (pH <7.12 and base deficit >12 mmol/L), both with Apgar scores of 2 at 1 minute and 9 at 5 minutes.

Drugs. No maternal medication had been given to the four fetuses that died in utero or to six of the eight that were acidemic (pH <7.12 and base deficit >12 mmol/L) on delivery. Multivariate analyses on data from the 38 women who received α-methyldopa as compared with the remainder, with consideration of gestational age, showed no effect on basal heart rate, FHR variation, or outcome. Similar analyses on the smaller number who received nifedipine also showed no effect.

Comment

We have sought to define more precisely the limits of FHR variation in the terminal traces of fetuses about to die because of increasing metabolic acidemia. Before the study began, we did not measure short-term variation but only the mean minute range (long-term variation). However, this index failed to detect a terminal trace with a sinusoidal pattern superimposed on a heart rate with very low short-term variation. Sinusoidal patterns occurred in one of nine terminal traces. This led us to examine measurements of short-term variation. Very-short-term FHR variation (literally beat-to-beat)

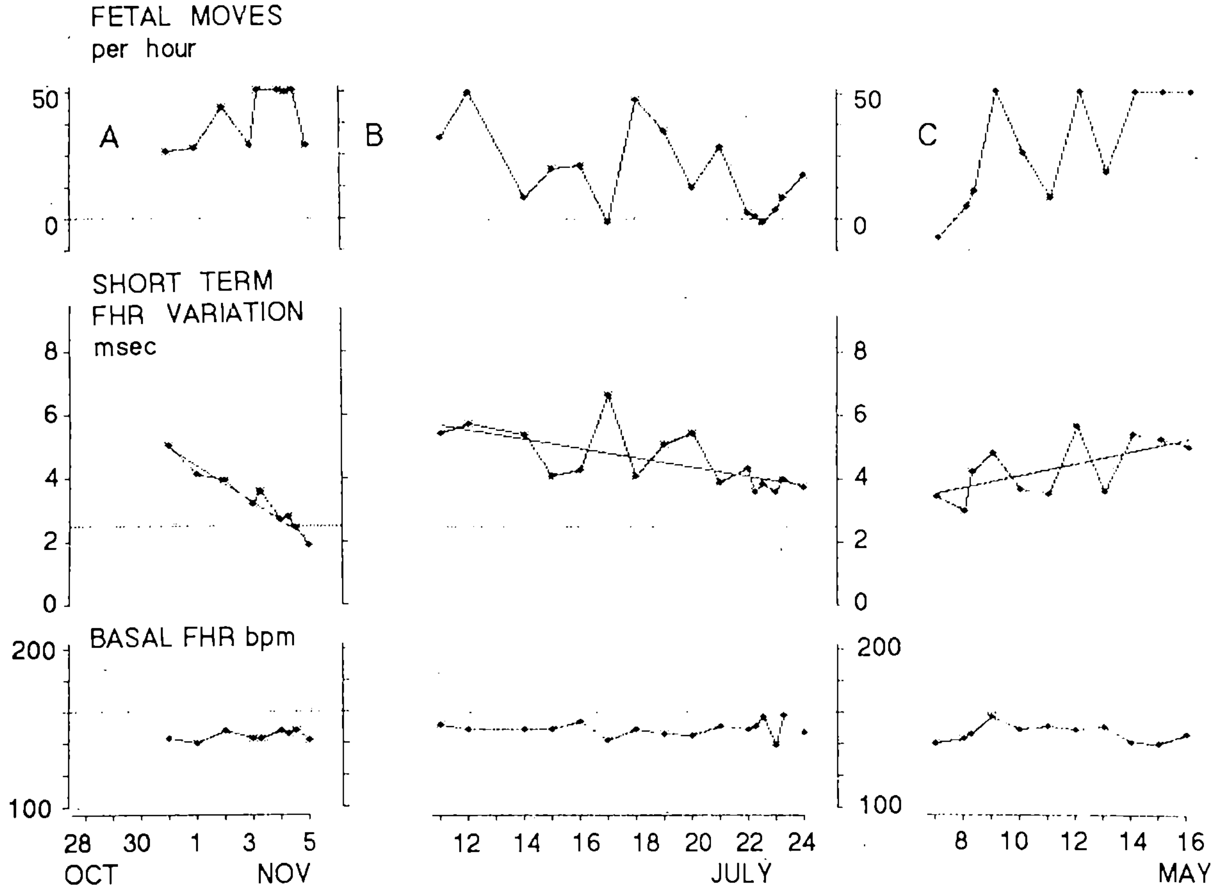

Fig. 3. Three synoptic displays of number of fetal movements per hour *(top)*, of short-term FHR variation *(middle)*, and of basal heart rate *(bottom)*. *Horizontal lines* are drawn at 10 movements per hour, short-term variation 2.5 milliseconds, and basal heart rate 160 beats/min. **A,** From pregnancy at 32 weeks with significant downward trend in variation (−0.58 msec/day; $p < 0.01$). **B,** Slower decline ($p < 0.01$) between 32 and 34 weeks. **C,** Significant upward trends in variation (+0.20 msec/day; $p < 0.02$) at 27 to 28 weeks' gestation; delivery was undertaken for maternal reasons. In no instance was there metabolic acidemia on delivery.

can be measured accurately from a scalp clip electrocardiogram in labor but not by ultrasonographic Doppler methods. Even from a scalp clip the normal value is low, only 2.1 ± 0.2 milliseconds[8] (mean pulse interval variation ± SE) or 1.98 ± 0.32 milliseconds[9] in two separate studies of normal fetuses during labor. This is at the limit of accuracy of measurement. The FHR cannot be detected reliably, between 26 and 34 weeks' gestation, by abdominal electrocardiogram. This precludes the accurate measurement of beat-to-beat variation. The problem is aggravated by the use of autocorrelation, a process that uses two successive pulse intervals to achieve an estimate of heart rate and so reduces (literally) the value of beat-to-beat FHR variation.[9]

We therefore defined a new measure of short-term variation as that between successive 3.75-second (1/16-minute) epochs during which valid FHR values are averaged (epoch-to-epoch variation). There are on average nine fetal pulses during each epoch at 144 beats/min. Epochs lasting 1/16 minute were first used in 1979 to reduce the data as much as possible (by a factor of 9) without compromising detection of brief changes in FHR from the baseline (small accelerations or decelerations) and to minimize the effect of signal loss. The epoch-to-epoch variation uses the least interval compatible with our data collection system, yet the mean value is sufficiently large to provide a good measure of short-term variation as it declines before death in utero.

The mean value of epoch-to-epoch variation for normal fetuses near term (corresponding to a long-term variation of 50 milliseconds, Fig. 2) is about 9 milliseconds. We have previously demonstrated that a mean minute range >30 milliseconds is associated with normal outcomes[17] and that it must fall to <20 milliseconds before there is evidence of chronic compensated hypoxemia at delivery by cesarean section in the absence of labor.[15] The limit of 20 milliseconds was used to define the population included in this study. A mean minute range of <15 milliseconds identified most of the compromised fetuses but with the important exception of five fetuses with slow sinusoidal FHR patterns, in whom the problem could be detected numerically only by measuring short-term variation. In a restricted sample of 44 cases with reduced short-term FHR variation, a final measurement of 2.5 milliseconds identified all terminal cases and three of the four preterminal cases with metabolic acidemia at birth. In five further fetuses encountered since the study was closed in 1987, short-term variation was 0.9 to 2.2 milliseconds within 24 hours of death; delivery was not undertaken because of extreme growth retardation and immaturity.

All such thresholds are empiric, widely used in medicine and necessary for convenience; they do not distinguish biologic differences in an absolute way but only define useful landmarks. In this study our purpose was to develop an objective measure of the terminal FHR trace. Given the small numbers, the constraints imposed by clinical interventions, and the fact that a few flat traces can have causes other than increasing metabolic acidemia, it should be clear that no claim can be made of an absolute measure of fetal respiratory status. However, no other noninvasive method can provide equivalent information about the fetus so objectively.

It has been considered that a reactive FHR is the most reliable guide to normality. We have shown that an alternative and better measure is identification of a short period in which the minute range is consistently high.[12, 17, 22] This is both more economical in time and more sensitive than the nonstress test. When combined with measurement of short-term FHR variation, it also follows that a contraction stress test is not required.

Gross reduction of FHR variation, unaccompanied by decelerations and with normal blood gas values at delivery, also has been described in some cases of anencephaly, after accidental fetal decerebration,[27] in two cases of fetal brain death,[28] and as a result of maternal administration of drugs. A similar situation was reported in fetal lambs after destruction of the brain above the cervical spinal cord, i.e., including the pons and medulla but not otherwise.[25] In these circumstances the baroreflex was lost. Thus identification of a very flat FHR trace is compatible with a number of diagnostic conclusions, of which the commonest (chronic, possibly compensated hypoxemia and incipient acidemia associated with fetal growth retardation and proteinuric preeclampsia) is now estimated to include 95%.

Our conclusions are provisional and their validity needs to be tested prospectively. The use of computerized analysis confers the major advantage of consistency in interpretation. Loss of FHR variation, which is impossible to estimate visually, needs to be extreme before fetal health is jeopardized.

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Maturation of the uterine cervix by repeated intracervical instillation of prostaglandin E_2

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An attempt was made to ripen the uterine cervix in 100 high-risk pregnancy patients (pregnancy between 34 to 41 weeks' gestation), with the use of intracervical instillations of 0.25 mg of prostaglandin E_2 mixed with a tylose gel. The maturation process was repeated every 48 hours. Forty-nine patients were delivered of infants after the first maturation and 51 patients required between two and nine instillations. In patients requiring multiple instillations, the mean delay between the first procedure and delivery was 9 ± 4 days (range, 2.4 to 16 days). Among the 59 nulliparous women, only 23 were delivered of infants after a single maturation and 36 required multiple maturations ($p < 0.02$). When the group of patients who were delivered of infants after a single maturation process was compared with the group requiring multiple maturations, no difference could be seen with regard to age, term of pregnancy, or Bishop cervical score at the time of inclusion in the study. The myometrial activity and the onset labor induced by prostaglandin E_2, were similar in both groups. Fetal heart rate decelerations occurred in 16.3% (8/49) of the patients with single maturations and in 17.6% (9/51) of the patients who required multiple maturations. The outcome of the pregnancy and the rate of cesarean sections (24% and 27%) were similar in both groups. No patients required cesarean sections because of failed induction of labor. Cervical ripening after repeated applications of 0.25 mg of prostaglandin E_2 seems to be safe for the fetus, providing that the patient is closely supervised. (AM J OBSTET GYNECOL 1991;165:523-8.)

Key words: Prostaglandin, maturation, cervix, pregnancy

Intracervical application of prostaglandin E_2 (PGE_2) is now advised when maturation of the uterine cervix is necessary for the induction of labor in high-risk pregnancies.[1] Usually, the recommended protocol consists of instilling a single dose of 0.5 mg of a PGE_2 gel into the cervix, followed 12 hours later, if regular uterine

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Table I. Indications for cervical maturation

Gestational hypertension	33
Preeclampsia	11
Fetal growth retardation	22
Postdate pregnancy	15
Insulin-dependent diabetes	12
FHR loss of variability	3
Rh disease	2
Addison's disease	1
Myasthenia gravis	1
Total No. of patients	100

contractions do not ensue, by the induction of labor with intravenous oxytocin.[2-6] Other application sites for PGE_2 gel have been tried, namely in the extraamniotic space or the posterior vaginal fornix,[7, 8] and larger doses of PGE_2 have been instilled into the cervix.[9, 10] However, in each of these previous attempts the use of the PGE_2 gel was limited to a single maturation of the cervix and if labor was not established in the mean time, induction was carried out 12 hours later.

Prospective multicentric randomized controlled trials[4, 11] and a double-blind controlled trial,[12] have clearly shown that a single dose of 0.5 mg of PGE_2 gel is superior to placebo in maturing the uterine cervix. However, the disadvantage with a single application of the PGE_2 gel is that when the maturing effect is insufficient, labor may be induced on a still unfavorable cervix 12 hours later. The failure rate in the induction of labor and the need of cesarean sections are then nearly as high as when the cervix has been ripened with placebo.[12]

Very few studies have attempted repeated maturations of the cervix when the first dose of PGE_2 appeared to be insufficient,[13, 14] and all of them were limited to a second application of the PGE_2 gel 6 or 12 hours after the first. Because the need for cervical maturation before induction of labor nearly always applies in high-risk pregnancies, the reaction of the endangered fetuses to repeated applications of PGE_2 is obviously a matter of concern.

Therefore this study was designed to test the effect of repeated maturations with PGE_2 gel on the fetus in high-risk pregnancies. The aim in all cases was to obtain a favorable cervix before the induction of labor. The outcome of 49 deliveries obtained after a single maturation was compared with the outcome of 51 pregnancies subjected to repeated applications of the PGE_2 gel.

Material and methods

A total of 100 high-risk pregnancy patients from our antenatal clinic were included in the study. The investigation was approved by the local Human Investigation Committee and informed consent was obtained. In all cases either the maternal or the fetal conditions justified induction of labor (Table I). Patients with breech presentations, premature rupture of membranes, previous cesarean sections, and uterine contractions were excluded from the study. All patients had a cervical Bishop score[15] less than 5 at the time of the first maturation.

All procedures were performed in the labor ward. Each patient was checked for cervical score and monitored over a period of 30 minutes to ensure that the fetal heart rate (FHR) was normal and that there were few or no uterine contractions (fewer than three per 30 minutes).

Cervical maturation was established by adding 0.25 mg of dinoprostone (Prostin E_2, Upjohn Co., Paris), to 3 ml of tylose gel prepared immediately before use. The patient was placed in a slight Trendelenburg position; the gel was gently instilled into the cervical canal with a size 8F plastic catheter, starting at the internal os and progressively withdrawing the catheter. Cardiotocography was maintained in the labor ward for at least 2 hours afterward, and was continued in cases of persistent uterine contractions or established labor.

The Bishop score was assessed by the same person, 2, 24, and 48 hours after instillation of the gel. Cardiotocography (duration, 30 minutes) was repeated every 12 hours until the following maturation procedure 48 hours later. The investigation was interrupted within the 48 hours after the first maturation for three reasons: onset of labor before the following maturation procedure; rapid deterioration of the fetal or maternal condition requiring prompt delivery (irrespective of the cervical status); improvement of the Bishop score to 6 or more, allowing a safe induction of labor with oxytocin.

With exception to the previous three circumstances, a second instillation of 0.25 mg of PGE_2 gel was given 48 hours after the first and was repeated every 48 hours thereafter under the same conditions. The onset of labor, an aggravation of the maternal or fetal condition requiring induction of labor, or an improved Bishop score up to at least 6 allowed a break in the sequence of repeated maturations of the cervix.

All the 100 patients who needed induction received a first application of the gel. Those who labored spontaneously, had a Bishop score greater than 6, or experienced maternal or fetal distress were included in the group with the single gel application and the remainder were in the group with multiple gel applications.

For each maturation the following parameters were recorded: maternal blood pressure, heart rate, and temperature. Cardiotocographic data were analyzed as follows: uterine contractions were recorded with a numeric score[16] as absent (0), mild and irregular (1), pain-

Table II. Termination of pregnancy after cervical maturation

No. of sequential maturations		*Spontaneous onset of labor*	*Induction of labor with oxytocin*						*Cesarean sections*		*Delay from first maturation to delivery (days)*
			Maternal indication			*FHR anomaly*					
M	*n*		*Hypertension*	*Ruptured membranes*	*Uterine contractions*	*Decelerations*	*Loss of variability*	*Favorable cervix*	*Acute fetal distress*	*Fetopelvic disproportion*	
1	49	13	5	1	4	3	3	20	10	2	0.67 (16.2 ± 4 hr)
2	28	5	2	2	1	2	1	15	2	6	2.4 ± 0.8
3	12	1	—	3	—	3	—	5	3	1	5.3 ± 1
4	3	1	—	—	—	—	—	2	—	—	6.6 ± 0.5
5	4	2	—	1	—	—	—	1	2	1	9.25 ± 0.8
6	2	—	—	1	—	—	—	1	—	—	11.1 ± 1
7	1	—	—	—	—	—	—	1	—	—	12
9	1	—	—	—	—	—	—	1	—	—	16
TOTAL		22	7	8	5	8	4	46	17	10	—

M, Sequential maturations; *n,* number of patients.

ful and irregular (2), or painful and regular (3); FHR decelerations were classified as absent, mild, moderate, or severe, according to their intensity and duration.[17]

Maturations were performed irrespective of the term of pregnancy. Fifteen patients had a term of pregnancy <37 weeks at the time of the first maturation, with three patients being at 34 weeks' gestation.

Statistical analysis. All the results were expressed as means with SD. Comparison of the means was carried out by analysis of variance. If the Fisher test was significant ($p < 0.05$), variables between the series were then compared with the Student t test (significant p value < 0.05).

Results

A total of 201 maturation procedures were performed on 100 patients: 49 were delivered of infants after one maturation and 51 required multiple maturations.

Termination of pregnancy. The mode of delivery, indication for induction of labor or cesarean section, and the mean delay between gel application and onset of labor are represented in Table II, according to the number of maturations applied.

Among the 51 patients who required multiple maturations, 12 patients had three, three patients had four, four patients had five, two patients had six, one patient had seven, and one patient had nine maturations.

No undesirable side effect of prostaglandins (i.e., nausea, vomiting, or diarrhea) was observed in any of the 201 maturation procedures performed.

Myometrial response to the PGE$_2$ gel. In 201 maturations, no myometrial activity was recorded in 30 patients.

In 130 patients, uterine contractions were exerienced but lasted <2 hours after the instillation of the gel. Table III indicates the score of the uterine contractions observed for each group of single or multiple maturations. Contractions produced moderate or severe FHR decelerations in six patients. Labor was immediately induced, resulting in three vaginal deliveries and three cesarean sections for persistent fetal distress (repetitive slowing of FHR and meconium-stained amniotic fluid). Type 3 uterine contractions were recorded for 11 patients and were resolved spontaneously. Myometrial activity also stopped spontaneously with no further consequences for the remaining 113 maturations.

In 41 maturations, myometrial activity lasted longer than 2 hours. In 13 patients, all with a single maturation, myometrial activity did not resolve and resulted in established labor. Labor was induced for five other patients because of persistent painful uterine contractions and for two patients because of FHR decelera-

Table III. Myometrial activity within 2 hours after a maturation

No. of maturations according to number of sequences		*Type of uterine contraction*			
M	*n*	*0*	*1*	*2*	*3*
1	49	10	29	16	4
2	56	24	20	9	3
3	36	7	26	3	—
4	12	6	6	—	—
5	20	9	8	1	2
6	12	9	1	—	2
7	7	4	3	—	—
9	9	2	7	—	—

M, Number of sequences; *n,* number of maturations; *0,* no uterine contraction; *1,* uterine contraction mild and irregular; *2,* uterine contraction painful and irregular; *3,* uterine contraction painful and regular.

Table IV. Comparison between patients with single and multiple cervical maturations

	*Single maturation**		*Multiple maturations†*	
	No.	*%*	*No.*	*%*
Nulliparas	23	46.9	36	70.5‡
Age (yr)	27.8 ± 5.9		27.4 ± 5.8	
Term of pregnancy at first maturation (wk)	38.8 ± 2.2		38.1 ± 1.8	
Bishop score at first maturation	2.7 ± 1.2		2.5 ± 1.1	
FHR decelerations after maturation	8	16.3	9	17.6
Labor induced for FHR deceleration	3	6.1	5	9.8
Labor induced for persistent uterine contractions	4	8.1	1	1.9
Labor induced for favorable cervix	20	40.8	26	50.9
Spontaneous onset of labor	13	26.5	9	17.6
Cesarean sections	12	24.4	15	29.4
Cesarean sections for acute fetal distress	10	20.4	7	13.7
Birth weight (gm)	3056 ± 620		2967 ± 704	
SGA <10th percentile	16	32.6	20	39.2
Mean 1 min Apgar score (range)	7.5 ± 2.4 (1-10)		7.3 ± 2.6 (1-10)	
Mean 5 min Apgar score (range)	8.9 ± 1.1 (6-10)		9.1 ± 0.8 (5-10)	

*n = 49.

†n = 51.

‡$p < 0.02$.

tions; all resulted in vaginal delivery. For the 21 remaining maturations, uterine contractions stopped spontaneously and no further consequences occurred in a period of 4 to 24 hours.

Cervical response to PGE_2 gel. For the 100 patients included in the study the Bishop score increased from 2.6 ± 0.9 on the day of the first maturation to 6.3 ± 1.3 at the induction or the spontaneous onset of labor. An improvement in the cervical score up to 6 allowed a safe induction of labor in 46 patients, 20 after a single maturation, and the remainder of patients after repeated maturations. In addition for 32 patients (16 in each group), labor had to be induced because of the maternal or fetal condition. Overall, 78 inductions of labor were performed, and all proceeded successfully to an established labor. Spontaneous onset of labor occurred in the remaining 22 patients.

Fetal response to PGE_2 gel. In 17 of 201 maturations and 14 of 100 patients, FHR decelerations occurred before the onset of labor. These decelerations were mild in 10 patients, moderate in four, and severe in three. They disappeared with lateral recumbency in nine patients. For eight patients they led to the induction of labor, resulting in four vaginal deliveries and four cesarean sections because of persistent fetal distress. For these 14 neonates the mean birth weight was 2217 ± 290 gm, 10 of whom had a birth weight below the 10th percentile. The mean 1- and 5-minute Apgar scores for these 14 neonates were 7.2 ± 2.1 (range, 2 to 10) and 8.6 ± 2.1 (range, 5 to 10), respectively.

For three patients FHR decelerations appeared after the first 2 hours of maturation (6, 9, and 47 hours, respectively), leading to conservative management in one case and induction of labor in two.

Comparison between the groups of single and multiple maturations (Table IV). There were 23 nulliparous women (46%) in the group with single maturation and 36 nulliparous women (70.5%) in the group with multiple maturations ($p < 0.02$). However, no difference could be seen between both groups when compared for age, term of pregnancy, or Bishop score at the time of inclusion in the study. FHR decelerations induced by the PGE_2 gel occurred in 16.3% of the patients (8/49) with one maturation, and 17.6% of the patients (9/51) with multiple maturations. The number of patients who required induction of labor because of FHR decelerations, persistent uterine contractions, or improved cervical score and the number of patients who experienced spontaneous labor after maturation were similar in both groups.

Twenty-seven patients had cesarean sections, 12 after a single maturation and 15 after repeated maturations. The number of cesarean sections for acute fetal distress was similar in both groups (10 and seven, respectively). The other cesarean sections (two and eight, respectively) were indicated for fetopelvic disproportion.

The outcome of pregnancy, birth weights, growth retardation below the 10th percentile, 1- and 5-minute Apgar scores, were not different between both groups of patients.

Comparison between nulliparas and multiparas (Table V). Twenty-six of the 41 multiparas (63.4%) were delivered of infants after one maturation, whereas only 23 of the 59 nulliparas (38.9%) were so ($p < 0.02$). Multiparas were older than nulliparas (30.9 ± 6.1 vs

Table V. Comparison between nulliparas and multiparas

	*Nulliparas**		*Multiparas†*	
	No.	*%*	*No.*	*%*
Single maturation	23	38.9	26	63.4‡
Age (yr)	25.3 ± 4.8		30.9 ± 6.1§	
Term of pregnancy at first maturation (wk)	38.4 ± 2.3		37.8 ± 1.9	
Bishop score at first maturation	2.7 ± 0.8		2.5 ± 0.9	
FHR decelerations after maturation	8	13.5	6	14.6
Labor induced for FHR deceleration	6	10.1	5	12.1
Labor induced for persistent uterine contractions	3	5	4	9.7
Labor induced for favorable cervix	28	47.4	16	39
Spontaneous onset of labor	15	25.4	9	21.9
Cesarean sections	17	28.8	10	24.3
Cesarean sections for acute fetal distress	11	18.6	6	14.6
Birth weight (gm)	2958 ± 620		3085 ± 704	
SGA <10th percentile	24	40.6	12	29.2
Mean 1 min Apgar score (range)	7.9 ± 2.1 (1-10)		7.3 ± 2.4 (1-10)	
Mean 5 min Apgar score (range)	9.2 ± 0.8 (8-10)		9 ± 0.9 (5-10)	

*$n = 59$.

†$n = 41$.

‡$p < 0.02$.

§$p < 0.001$.

25.3 ± 4.8; $p < 0.001$). However, when compared for term of pregnancy, Bishop score at the time of the first maturation, or the outcome of the pregnancy, no difference could be shown between nulliparas and multiparas.

Comment

When labor is induced 12 hours after cervical ripening with a local application of 0.5 mg of PGE_2, failed induction ranges between 13.5% and 16%[4, 12] and can possibly lead to avoidable cesarean sections. Higher doses of PGE_2 increase the liability of unwanted uterine contractions or hyperstimulation.[9, 10] Therefore there is a need for a new approach to the local application of PGE_2, for example, sequential intracervical instillations. Because this study does not include a controlled series, the superiority of repeated PGE_2 low-dose applications over other methods of cervical ripening cannot be assessed, but the potential hazards, particularly with regard to the fetus, can be evaluated.

The main finding of our study is that repeated maturations do not provoke any greater risk of distress for the fetus or the neonate than a single maturation, providing that they are closely supervised after each maturation. In the 201 maturations performed, 8.4% (17/201) were complicated by FHR decelerations, which is also the rate reported after cervical ripening with 0.5 mg of PGE_2 and after intracervical instillation of a placebo gel.[11] However, the frequency of FHR decelerations was not different for the patients with one maturation (16.3%) or multiple maturations (17.6%). The outcome of pregnancy was also similar in both groups of patients, and in spite of a population with high-risk pregnancies, no particular detrimental effect of multiple maturations was recorded for the neonates, even after as many as seven or nine repeated maturations.

It is surprising that the reduction of the dose of PGE_2 from 0.5 mg to 0.25 mg did not prevent myometrial activity during cervical ripening. Cervical ripening with 0.5 mg of PGE_2 induces 4% to 12% incidence of myometrial hyperstimulation,[2, 5, 6, 11, 12] and entails 0.5% to 3% cesarean sections for acute fetal distress. Myometrial hyperstimulation occurs more frequently after extraamniotic application of the PGE_2 gel[4] and has been reported after cervical application of a placebo gel.[4, 11, 12] Granström et al.[18] found that proper intracervical instillation of 0.5 mg of PGE_2 gel, in contrast with extraamniotic or vaginal application, induces cervical ripening without significant myometrial activity. Our results are at odds with the findings of Granström et al. because after intracervical instillation of only 0.25 mg of PGE_2, uterine contractions occurred in 85% of the maturations (171/201) and in 20% of the patients myometrial activity persisted beyond 2 hours. However, tocolysis was never used because β-sympathomimetic agents administered with PGE_2 may lead to untoward effects.[11]

The aim of repeated maturations with PGE_2 was to obtain in all instances a favorable cervix before planned induction of labor. After application of 0.5 mg of PGE_2, labor is established before oxytocin induction in 38.5% of the patients.[12] In this study, the PGE_2 gel acted beyond a simple ripening effect of the cervix and was sufficient enough to induce labor in 22% of the patients. Whereas failed induction of labor occurred in 13.5% to 16% of the patients after maturation with a single dose of 0.5 mg of PGE_2,[4, 12] no failure was recorded for the 78 inductions of labor in this study and all proceeded successfully to an established labor with a cervical dilatation of ≥3 cm. It is worth noting that 15% of our patients had their first maturation before 37 weeks of amenorrhea (three as early as 34 weeks), whereas all previous studies[1-14] dealt with term pregnancies.

A puzzling finding of this study was the difference in sensitivity of the cervix and myometrium to PGE_2 between the 49 patients who were delivered of infants after a single maturation and the 51 patients who required multiple maturations. Of course there were significantly more nulliparous patients in the group of multiple maturations. However, both groups (single and multiple maturations) along with the two groups of nulliparas and multiparas were otherwise similar with regard to the term of pregnancy and to the Bishop score at the time of the first maturation. Myometrial contractility and cervical distensibility are subject to concordant endocrine regulation, involving estrogen: progestogen ratios, prostanoids, and relaxin.[19, 20] Maturation of the cervix implies changes in the structure and biochemistry of the connective tissue, namely dissolution of the collagen fibrils, diminution of the dermatan sulfate, and increase in hyaluronic acid and water content. Therefore induction of labor probably needs to be supported by more accurate means to measure cervical compliance and distinguish parity than the current clinical evaluation.

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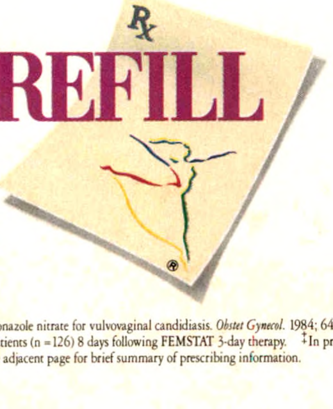

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*Micrographs on file, Syntex Laboratories, Inc. †Microbiological cure in 95% of patients (n = 126) 8 days following FEMSTAT 3-day therapy. ‡In pregnant patients (second and third trimesters only), therapy should be extended to 6 days. Please see adjacent page for brief summary of prescribing information.
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Information for the Patient: Do not discontinue prematurely during menstruation or because of symptomatic relief.

Carcinogenesis: Animal studies have not been done.

Mutagenesis: Mutagenicity studies were negative.

Impairment of Fertility: Animal studies showed no impairment of fertility.

Pregnancy Category C: Adverse effects were noted in animals treated with high oral doses. No studies were done in women during first trimester. Patients in the second or third trimester have shown no adverse effects attributable to the drug.

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Pediatric Use: Safety and efficacy have not been established.

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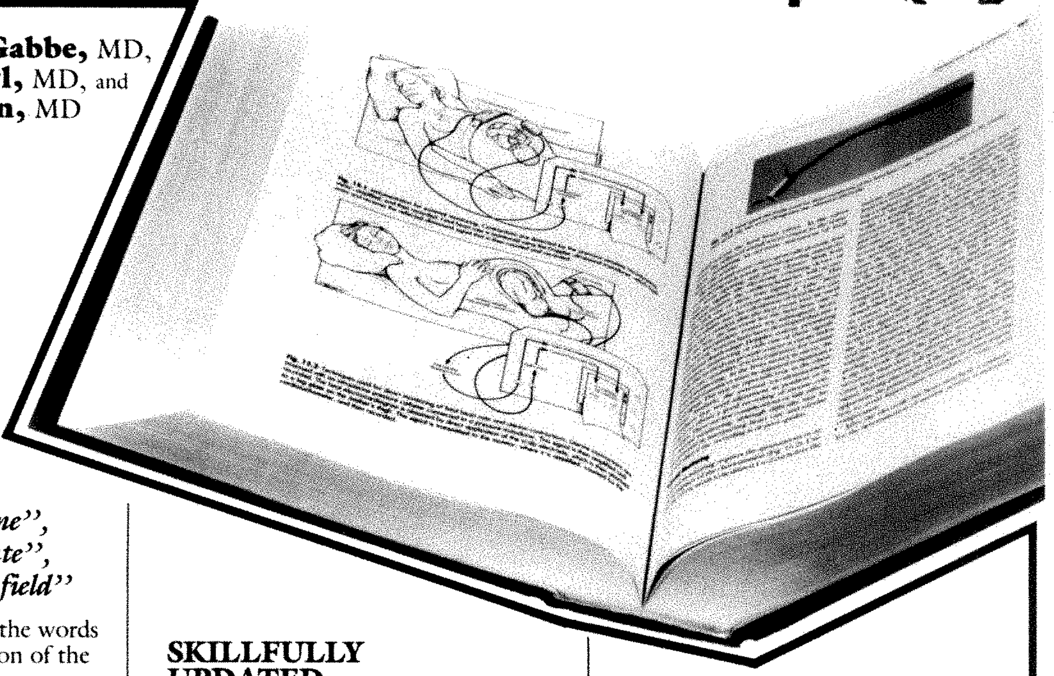

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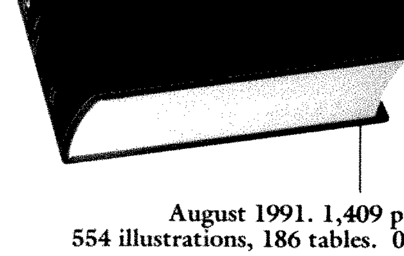

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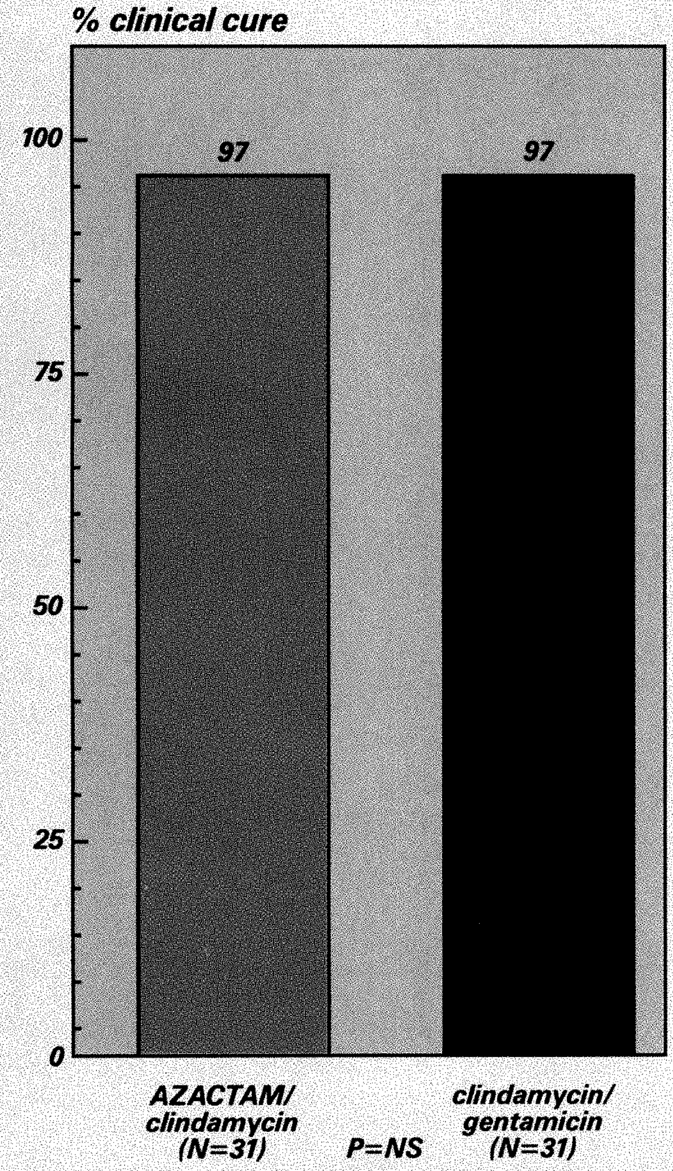

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†Although a useful guide, in vitro data do not necessarily correlate with therapeutic outcome.

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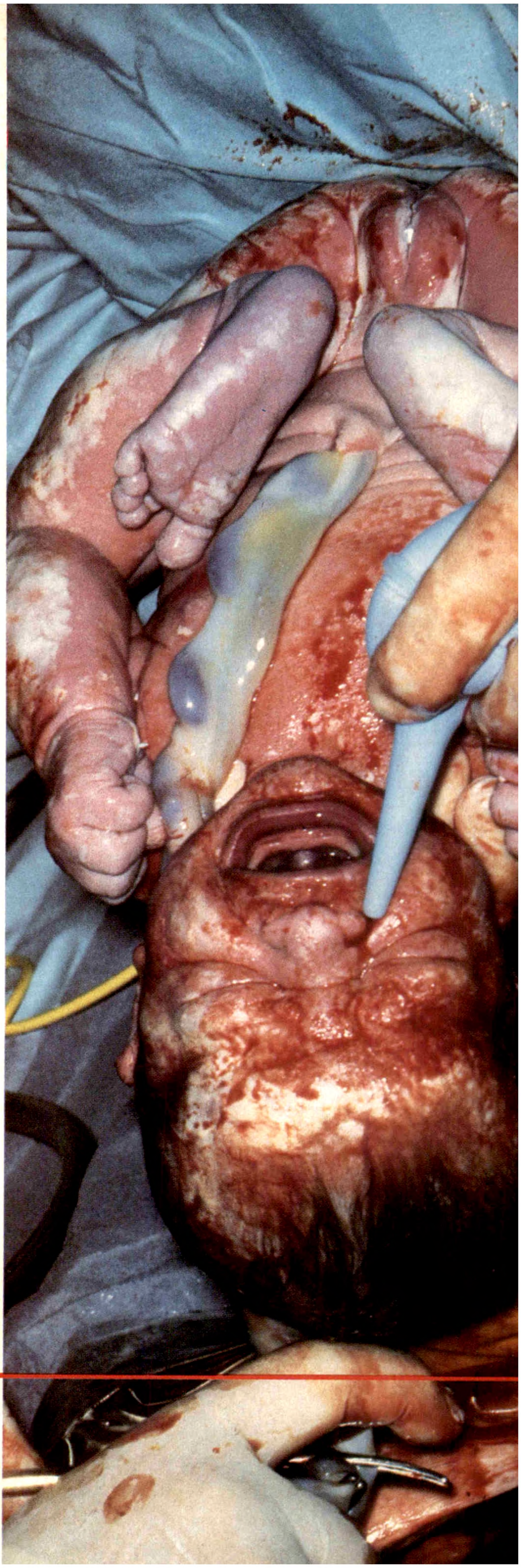

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AZACTAM For Injection is a sterile, nonpyrogenic, sodium-free, white to yellowish-white lyophilized cake, containing approximately 780 mg arginine per gram of aztreonam for intramuscular or intravenous use following constitution. Aqueous solutions of the product have a pH in the range of 4.5-7.5.

INDICATIONS AND USAGE–Before initiating treatment with AZACTAM, appropriate specimens should be obtained for isolation of the causative organism(s) and for determination of susceptibility to aztreonam. Treatment with AZACTAM may be started empirically before results of the susceptibility testing are available; subsequently, appropriate antibiotic therapy should be continued.

AZACTAM For Injection is indicated for the treatment of the following infections caused by susceptible gram-negative microorganisms: **Urinary Tract Infections** (complicated and uncomplicated), including pyelonephritis and cystitis (initial and recurrent) caused by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Enterobacter cloacae, Klebsiella oxytoca*, Citrobacter* species* and *Serratia marcescens**. **Lower Respiratory Tract Infections,** including pneumonia and bronchitis caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae, Proteus mirabilis, Enterobacter* species and *Serratia marcescens**. **Septicemia** caused by *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis*, Serratia marcescens** and *Enterobacter* species. **Skin and Skin-Structure Infections,** including those associated with postoperative wounds, ulcers and burns caused by *Escherichia coli, Proteus mirabilis, Serratia marcescens, Enterobacter* species, *Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Citrobacter* species*. **Intra-abdominal Infections,** including peritonitis caused by *Escherichia coli, Klebsiella* species, including *K. pneumoniae, Enterobacter* species including *E. cloacae*, Pseudomonas aeruginosa, Citrobacter* species* including *C. freundii** and *Serratia* species* including *S. marcescens**. **Gynecologic Infections,** including endometritis and pelvic cellulitis caused by *Escherichia coli, Klebsiella pneumoniae*, Enterobacter* species* including *E. cloacae**, and *Proteus mirabilis**.

AZACTAM is indicated for adjunctive therapy to surgery in the management of infections caused by susceptible organisms, including abscesses, infections complicating hollow viscus perforations, cutaneous infections and infections of serous surfaces. AZACTAM is effective against most of the commonly encountered gram-negative aerobic pathogens seen in general surgery.

Concurrent Therapy–Concurrent initial therapy with other antimicrobial agents and AZACTAM is recommended before the causative organism(s) is known in seriously ill patients who are also at risk of having an infection due to gram-positive aerobic pathogens. If anaerobic organisms are also suspected, therapy should be initiated using an antianaerobic agent concurrently with AZACTAM. Certain antibiotics (e.g., cefoxitin, imipenem) may induce high levels of beta-lactamase *in vitro* in some gram-negative aerobes such as *Enterobacter* and *Pseudomonas* species, resulting in antagonism to many beta-lactam antibiotics including aztreonam. These *in vitro* findings suggest that such beta-lactamase inducing antibiotics not be used concurrently with aztreonam. Following identification and susceptibility testing, appropriate antibiotic therapy should be continued.

CONTRAINDICATIONS–Aztreonam is contraindicated in patients with known allergy to this antibiotic.

WARNINGS–Pseudomembranous colitis has been reported with nearly all antibacterial agents, including aztreonam, and may range in severity from mild to life-threatening. Therefore, it is important to consider this diagnosis in patients who present with diarrhea subsequent to the administration of antibacterial agents.

Treatment with antibacterial agents alters the normal flora of the colon and may permit overgrowth of clostridia. Studies indicate that a toxin produced by *Clostridium difficile* is one primary cause of "antibiotic-associated colitis."

After the diagnosis of pseudomembranous colitis has been established, therapeutic measures should be initiated. Mild cases of pseudomembranous colitis usually respond to drug discontinuation alone. In moderate to severe cases, consideration should be given to management with fluids and electrolytes, protein supplementation, and treatment with an oral antibacterial drug effective against *C. difficile* (e.g., vancomycin). Careful inquiry should be made for a history of hypersensitivity reaction to any antibiotic or other drugs. Antibiotics should be given with caution to any patient who has had some form of allergy, particularly to drugs. It is recommended that patients who have had immediate hypersensitivity reactions (e.g., anaphylactic or urticarial) to penicillins and/or cephalosporins should be followed with special care. If an allergic reaction to aztreonam occurs, discontinue the drug and institute supportive treatment as appropriate (e.g., maintenance of ventilation, pressor amines, antihistamines, corticosteroids). Serious hypersensitivity reactions may require epinephrine and other emergency measures.

PRECAUTIONS–General: In patients with impaired hepatic or renal function, appropriate monitoring is recommended during therapy. If an aminoglycoside is used concurrently with aztreonam, especially if high dosages of the former are used or if therapy is prolonged, renal function should be monitored because of the potential nephrotoxicity and ototoxicity of aminoglycoside antibiotics. The use of antibiotics may promote the overgrowth of nonsusceptible organisms, including gram-positive organisms and fungi. Should superinfection occur during therapy, appropriate measures should be taken.

Carcinogenesis, Mutagenesis, Impairment of Fertility–Carcinogenicity studies in animals have not been performed. Genetic toxicology studies performed *in vivo* and *in vitro* with aztreonam in several standard laboratory models revealed no evidence of mutagenic potential at the chromosomal or gene level. Two-generation reproduction studies in rats at daily doses up to 20 times the maximum recommended human dose, prior to and during gestation and lactation, revealed no evidence of impaired fertility. There was a slightly reduced survival rate during the lactation period in the offspring of rats that received the highest dosage, but not in offspring of rats that received five times the maximum recommended human dose.

Pregnancy–Pregnancy Category B: Aztreonam crosses the placenta and enters the fetal circulation. Studies in pregnant rats and rabbits, with daily doses up to 15 and 5 times, respectively, the maximum recommended human dose, revealed no evidence of embryo- or fetotoxicity or teratogenicity. No drug induced changes were seen in any of the maternal, fetal or neonatal parameters that were monitored in rats receiving 15 times the maximum recommended human dose of aztreonam during late gestation and lactation. There are no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, aztreonam should be used during pregnancy only if clearly needed.

Nursing Mothers–Aztreonam is excreted in breast milk in concentrations that are less than 1% of concentrations determined in simultaneously obtained maternal serum; consideration should be given to temporary discontinuation of nursing and use of formula feedings.

Pediatric Use–Safety and effectiveness have not been established in infants and children.

ADVERSE REACTIONS–Local reactions such as phlebitis/thrombophlebitis following IV administration, and discomfort/swelling at the injection site following IM administration occurred at rates of approximately 1.9% and 2.4%, respectively. Systemic reactions (considered to be related to therapy or of uncertain etiology) occurring at an incidence of 1 to 1.3% include diarrhea, nausea and/or vomiting, and rash. Reactions occurring at an incidence of less than 1% are listed within each body system in order of decreasing severity; *Hypersensitivity*–anaphylaxis. *Hematologic*–pancytopenia, neutropenia, thrombocytopenia, anemia, leukocytosis, thrombocytosis. *Gastrointestinal*–abdominal cramps; rare cases of *C. difficile*-associated diarrhea, including pseudomembranous colitis, or gastrointestinal bleeding have been reported. Onset of pseudomembranous colitis symptoms may occur during or after antibiotic treatment (see WARNINGS). *Dermatologic*–purpura, erythema multiforme, urticaria, exfoliative dermatitis, petechiae, pruritus, diaphoresis. *Cardiovascular*–hypotension, transient ECG changes (ventricular bigeminy and PVC). *Respiratory*–one patient experienced flushing, chest pain, and dyspnea. *Hepatobiliary*–hepatitis, jaundice. *Nervous System*–seizure, confusion, vertigo, paresthesia, insomnia, dizziness. *Musculoskeletal*–muscular aches. *Special Senses*–tinnitus, diplopia, mouth ulcer, altered taste, numb tongue, sneezing and nasal congestion, halitosis. *Other*–vaginal candidiasis, vaginitis, breast tenderness. *Body as a Whole*–weakness, headache, fever, malaise.

Adverse Laboratory Changes–Those reported without regard to drug relationship during clinical trials were: *Hepatic*–elevations of AST (SGOT), ALT (SGPT), and alkaline phosphatase; signs or symptoms of hepatobiliary dysfunction occurred in less than 1% of recipients (see above). *Hemic*–increases in prothrombin and partial thromboplastin times, eosinophilia, positive Coombs test. *Renal*–increases in serum creatinine.

OVERDOSAGE–If necessary, aztreonam may be cleared from the serum by hemodialysis and/or peritoneal dialysis.

DOSAGE AND ADMINISTRATION–Dosage adjustments are recommended for patients with impaired renal function. In elderly patients, estimates of creatinine clearance should be obtained and appropriate dosage modifications made if necessary.

HOW SUPPLIED–AZACTAM For Injection (Aztreonam For Injection)–Lyophilized–is supplied in single-dose 15 mL vials containing **500 mg,** or **1 g**/vial; in single-dose 30 mL vials containing **2 g**/vial; and in single-dose 100 mL intravenous infusion bottles containing **500 mg** or **1 g** or **2 g**/bottle.

Consult package insert before prescribing AZACTAM (aztreonam). (J4-231C)

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*Efficacy for this organism in this organ system was studied in fewer than ten infections.

References: *1. Greenberg RN, Reilly PM, Weinandt WJ, et al: Comparison trial of clindamycin with aztreonam or gentamicin in the treatment of postpartum endometritis. Clin Ther 10(1):36-39, 1987.* ***2.*** *Gibbs RS, Blanco JD, Lipscomb KA, et al: Aztreonam versus gentamicin, each with clindamycin, in the treatment of endometritis. Obstet Gynecol 65:825-829, 1985.* ***3.*** *Henry SA: Overall clinical experience with aztreonam in the treatment of obstetric-gynecologic infections. Rev Infect Dis 7(suppl 4):S703-S708, 1985.*

SQUIBB

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Hypoplastic left heart syndrome: Prenatal diagnosis, clinical profile, and management

Danielle M. Blake, MD, Joshua A. Copel, MD, and Charles S. Kleinman, MD
New Haven, Connecticut

We diagnosed hypoplastic left heart syndrome, generally regarded as a lethal congenital heart defect, by fetal echocardiography in 20 pregnancies in the last 5 years. The clinical profile, management, and outcome of these pregnancies were reviewed. We found a 40% association of karyotype and extracardiac malformations. Elective abortion was performed in nine pregnancies. Two of seven live-born babies had early neonatal assessment and intervention as a result of in utero diagnosis and counseling. Prolonged survival was achieved in both infants. We conclude that prenatal diagnosis of the hypoplastic left heart syndrome necessitates complete evaluation of the fetus for associated genetic and extracardiac malformations. Prenatal diagnosis of this defect provides opportunities for in depth counseling of parents and obtaining informed consent for either postnatal intervention or nonintervention before the medical and emotional complexities associated with the neonatal intensive care setting are encountered. (AM J OBSTET GYNECOL 1991;165:529-34.)

Key words: Hypoplastic left heart syndrome, prenatal diagnosis, congenital heart disease

Hypoplastic left heart syndrome is a congenital heart defect that ranges in severity from critical aortic stenosis with nearly normal left ventricular and mitral valve dimensions to the most severe form in which both aortic and mitral valves are atretic and the left ventricle is absent or extremely underdeveloped.[1] It is the most common cause of death from heart disease during the first week of life. The greatest risk of dying occurs on day 3, at the time of ductal closure.[2,3] If left untreated, it is a lethal cardiac malformation.[4]

The prenatal diagnosis of hypoplastic left heart syndrome by fetal echocardiography has recently been well documented.[5-7] The ability to diagnose this and other structural heart defects in utero is a major contribution of high-resolution ultrasonography to obstetric care. The antenatal diagnosis of hypoplastic left heart syndrome mandates counseling of the parents; it affects further evaluation and management plans during the remainder of the pregnancy and during postnatal care of the neonate. Although the in utero diagnosis of hypoplastic left heart syndrome has been reported and management of the neonate with this defect is well described,[2,4] a comprehensive review of the clinical profile, management, and outcome of pregnancies diagnosed prenatally is lacking. In this article we present our center's 5-year experience with the prenatal diagnosis of hypoplastic left heart and review the characteristics, management, and outcome of the affected pregnancies.

Material and methods

All fetal echocardiograms obtained in the Fetal Cardiovascular Center at Yale University School of Medicine between January 1986 and December 1990 were reviewed. Indications for referral were factors known to be associated with increased risk for congenital heart disease in the fetus (Table I). At the time of study all patients underwent a detailed ultrasonographic examination followed by fetal echocardiography with 3.5 or 5.0 MHz transducers (model 77020AC, Hewlett-Packard, Andover, Mass.; Ultramark 9, Advanced Technology Laboratories, Bothell, Wash.).

Fetal echocardiograms included two-dimensional views of the four chambers, long- and short-axis views of the ventricular outflow tracts and great arteries, and aortic and ductal arch views. Two-dimensional real-time examination was supplemented by pulsed and color flow Doppler as these systems became available.

Pregnancies complicated by the prenatal diagnosis of hypoplastic left heart syndrome composed the study population. Scans were performed by a physician experienced in fetal cardiac ultrasonography and recorded on videotape for future review. Follow-up information was obtained from clinical or autopsy records from Yale–New Haven Hospital or by contacting the referring physicians. Parents were advised of the echocardiographic findings at the time of diagnosis. Management options for the pregnancy and postnatal period were explained. These included continuation of the pregnancy with either neonatal cardiac

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From the Fetal Cardiovascular Center, Departments of Obstetrics and Gynecology, Pediatrics, and Diagnostic Imaging, Yale University School of Medicine.
Received for publication December 27, 1990; revised March 11, 1991; accepted March 22, 1991.
Reprint requests: Joshua A. Copel, MD, Section of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510.
6/1/29785

Table I. Indications for fetal echocardiography

- Familial risk
  - Previous child with congenital heart disease
  - Father with congenital heart disease
  - Genetic syndromes (Noonan, tuberous sclerosis)
- Maternal risk
  - Congenital heart disease
  - Diabetes
  - Teratogen exposure
- Fetal risk
  - Suspicious four-chamber view
  - Extracardiac malformation
  - Chromosomal abnormality
  - Nonimmune hydrops
  - Arrhythmia

transplantation[8] or performance of a Norwood procedure[9] or nonintervention with compassionate care of the baby after birth.[10] Patients were informed that termination of pregnancy was an option at <24 weeks' gestation.

The indication for the study, the gestational age at diagnosis, associated extracardiac abnormalities, and fetal karyotype were reviewed. Pregnancy outcome was categorized as elective abortion or continuation of pregnancy resulting in either an intrauterine fetal death or a live-born infant. Pregnancies that continued to viability had the neonatal course reviewed with regard to intervention or nonintervention and survival. Autopsy records were reviewed where available.

Results

Between 1986 and 1990 we diagnosed hypoplastic left heart syndrome in 20 fetuses. The most common indication for referral for fetal echocardiography among this group of patients was an abnormal four-chamber view on general obstetric ultrasonographic examination (Tables II and III).

The diagnosis of hypoplastic left heart syndrome was made by the observation of a diminutive left ventricle and left atrium on the four-chamber view, with an enlarged right ventricle and right atrium. Mitral and aortic valve leaflet motion was absent or minimal. An abnormally narrowed aortic root and ascending aorta seen on the long axis great vessel view bordered by an enlarged pulmonary artery also suggested the diagnosis.

Color flow Doppler was helpful in establishing the diagnosis of hypoplastic left heart syndrome in 10 of the 15 cases in which it was used. Lack of blood flow into the left ventricle consistent with mitral valve atresia was seen in five cases (6, 7, 9, 16, and 20). Four cases (1, 2, 14, and 17) were associated with abnormal flow at the foramen ovale, with shunting from the left to the right atrium. At the site of entry into the descending aorta blood flow from the ductus arteriosus was observed to branch, flowing forward into the descending aorta and retrograde into the ascending aorta in five cases (1, 6, 16, 17, and 20). Forward flow into the ascending aorta was visualized in two cases (11 and 14). In 15 cases classic hypoplastic left heart syndrome, with mitral and aortic atresia, was diagnosed prenatally. A variant of this syndrome was diagnosed in an additional five cases (7, 8, 11, 14, and 18).

Fourteen patients had the diagnosis made before viability (Table II). Associated extracardiac abnormalities were identified prenatally in six fetuses. Four fetuses had cystic hygromas with associated nonimmune hydrops in three. The remaining abnormalities included a two-vessel cord and a cleft palate. Abnormal karyotypes were found in three fetuses. The karyotype abnormalities were 45,X in two fetuses with cystic hygromas and nonimmune hydrops and trisomy 13 in the fetus with cleft palate. Elective termination of pregnancy was performed in nine of the pregnancies diagnosed before viability. The remaining five patients decided to continue pregnancy. Three of these pregnancies resulted in intrauterine fetal death. One death occurred in a twin gestation with one affected fetus and a normal cotwin. The affected twin had trisomy 13 syndrome and a posterior cleft palate on ultrasonography; this twin died at 26 weeks' gestation. The other intrauterine deaths occurred at 22 weeks 5 days and at 24 weeks in two singleton pregnancies, one with isolated hypoplastic left heart syndrome and the other with cystic hygroma and a 45,X karyotype, respectively. Viable newborns were delivered at term in the remaining two pregnancies. Neither had any postnatal surgical intervention. The ages at death were 2 and 8 days. In 10 of these 14 cases either autopsy or postnatal echocardiography was done after completion of the pregnancy. In nine of these 10 cases further evaluation confirmed the prenatal diagnosis of hypoplastic left heart syndrome. The fetal heart in case 13 was grossly hypoplastic at autopsy, weighing only 0.9 gm. Chamber size and other structural dimensions could not be obtained because of the extremely small size of the heart. Follow-up studies were not done in cases 1, 7, 9, and 10.

Six patients had cardiac diagnosis made after 24 weeks (Table III). None of these six fetuses had an abnormal karyotype. Extracardiac abnormalities were present in two fetuses. One fetus had a two-vessel cord, and the other had an omphalocele. There was a single intrauterine death at 30 weeks' gestation. Expectant management and compassionate care had been decided on after the prenatal diagnosis of hypoplastic left heart syndrome had been made. However, cesarean delivery was performed in the setting of preterm labor and malpresentation. Autopsy confirmed mitral-aortic atresia with left ventricular hypoplasia. The only twin gestation in this group involved an affected fetus and a normal

Table II. Diagnosis of hypoplastic left heart syndrome before 24 weeks' gestation

Case No.	*Gestational age at diagnosis (wk)*	*Indication*	*Extracardiac anomaly*	*Karyotype*	*Pregnancy outcome*
1	22	Abnormal four chambers	—	46,XY	IUFD at 22.5 wk
2	20	Abnormal four chambers	Two-vessel cord	—	TAB
3	21	Abnormal four chambers Diabetes	—	46,XX	TAB
4	19	Anomaly	Cystic hygroma	Normal	Live-born; died on day 8
5	23.5	Abnormal four chambers	—	Normal	Live-born; died on day 2
6	21	Abnormal four chambers	—	46,XX	TAB
7	20	Anomaly Nonimmune hydrops	Cystic hygroma	45,XO	IUFD at 24 wk
8	19	Anomaly Nonimmune hydrops	Cystic hygroma	—	TAB
9	22	Abnormal karyotype, twin B	Posterior cleft palate	Trisomy 13	IUFD at 26 wk; macerated
10	22	Abnormal four chambers Elevated MSAFP Previous CHD	—	46,XY	TAB
11	22	Abnormal four chambers Previous HLHS	—	—	TAB
12	17	Abnormal four chambers	—	46,XY	TAB
13	20	Anomaly Nonimmune hydrops	Cystic hygroma	45,XO	TAB
14	23	Abnormal four chambers	—	Normal	TAB

IUFD, Intrauterine fetal death; *TAB*, elective abortion; *MSAFP*, maternal serum α-fetoprotein; *CHD*, congenital heart defect, *HLHS*, hypoplastic left heart syndrome.

Table III. Diagnosis of hypoplastic left heart syndrome after 24 weeks' gestation

Case No.	*Gestational age at diagnosis (wk)*	*Scan indication*	*Extracardiac anomaly*	*Karyotype*	*Outcome*
15	28	Abnormal four chambers Previous CHD	—	46,XY	Live-born
16	26	Abnormal four chambers Twin B, nonimmune hydrops	—	46,XX	Live-born; died on day 6
17	26.5	Abnormal four chambers	Two-vessel cord Hydramnios	46,XY	Stillborn
18	36	Abnormal four chambers	—	46,XX	Live-born
19	34	Anomaly	Omphalocele	46,XX	Live-born; died on day 1
20	29	Low MSAFP Abnormal four chambers	—	46,XX	Live-born; died on day 2

CHD, Congenital heart defect; *MSAFP*, maternal serum α-fetoprotein.

cotwin (case 16). Digoxin was administered to the mother with subsequent improvement in fetal hydrops and polyhydramnios in the affected twin, which had contributed to preterm labor. A noninterventional approach had been planned for the affected twin. Postnatal echocardiography after a term delivery confirmed the prenatal diagnosis. Neonatal death occurred on day 5.

Live-born infants were delivered in the remaining four pregnancies. Two of these babies had early postnatal intervention after confirmatory neonatal echocardiograms. In case 15 the infant underwent a Norwood procedure and currently is alive, having achieved appropriate developmental milestones at 2½ years. In case 18 the fetus was diagnosed prenatally as having a variant of hypoplastic left heart syndrome and postnatally underwent multiple cardiac surgical procedures on day 1, at 4 months, and at 18 months with ultimate death of the infant at 2½ years of intractable congestive heart failure. The remaining two live-born infants died on the first day of life. In case 19 the infant died within minutes of birth and had neither postnatal echocardiography nor autopsy. Case 20 had the cardiac diagnosis confirmed by postnatal echocardiographic study before death of the infant on day 2.

Overall, the outcomes of these 20 pregnancies included nine elective abortions, four intrauterine deaths, and seven live births. Neonatal death occurred between days 1 and 8 in five babies. Prolonged survival was achieved in two newborns who underwent early surgical intervention. The sole survivor at this time is a 2½-year-old child who underwent a Norwood procedure.

Comment

This series of cases demonstrates a poor outcome for the majority of pregnancies with the in utero diagnosis of hypoplastic left heart syndrome. A similar poor prognosis for pregnancies with prenatal diagnosis of a variety of congenital heart defects was described by Crawford et al.,[11] who reported only 17% survival of offspring of continuing pregnancies. Associated extracardiac or chromosomal anomalies occurred in 46% of cases with cardiac defects diagnosed prenatally. In this series of cases there were nine perinatal deaths among 11 continuing pregnancies. The two cases of prolonged survival both were subsequent to postnatal surgery. If elective abortions are included, the mortality figure is increased (18 of 20 pregnancies). Forty percent of cases diagnosed prenatally in our series had associated extracardiac or karyotype abnormalities. This is consistent with the data reported by Crawford et al. for prenatally diagnosed heart defects in general; however, it is considerably increased above the 28% frequency of genetic and major extracardiac abnormalities associated with hypoplastic left heart syndrome reported by Natowicz et al.[12] from pediatric autopsy cases and the 11% association reported by Morris.[5]

Series with prenatal diagnosis include cases of extracardiac abnormalities that underwent elective abortions and intrauterine deaths that may not be included in pediatric statistics and so explain the apparent discrepancy. Consistent with neonatal data,[13] the presence of Turner's syndrome in newborns with left-sided heart disease was cytogenetically confirmed in two of the four cases with in utero diagnosis of hypoplastic left heart syndrome and cystic hygromas. All three abnormal karyotypes were found in previable fetuses, two of which died in utero. No fetus with a diagnosis made after 24 weeks had an abnormal karyotype. Three of five continuing pregnancies diagnosed at <24 weeks had intrauterine deaths, compared to one of six pregnancies with a diagnosis made after 24 weeks. Our observations are consistent with poor in utero survival of cytogenetically abnormal fetuses with a diagnosis made early in pregnancy.[14] Abnormal cytogenetic studies may alter counseling regarding recurrence risks for future pregnancies, should features suggestive of a genetic syndrome be identified. Karyotype abnormalities and severe extracardiac malformations also may alter candidacy for postnatal surgery. These data are consistent with our previous suggestion that complete prenatal evaluation includes cytogenetic study of a pregnancy with a diagnosis of hypoplastic left heart syndrome.[15]

Prenatal diagnosis of hypoplastic left heart syndrome affords the opportunity for early intervention in the postnatal course of the neonate. Hypoplastic left heart syndrome renders the neonate dependent on persistent ductal patency to provide systemic blood flow; therefore prenatal diagnosis of hypoplastic left heart syndrome allows early postnatal confirmation of the diagnosis, with subsequent administration of prostaglandin E_1 infusion to maintain ductal patency before significant deterioration of the neonate. In addition, timely institution of ventilatory support can be initiated.[2, 4] If cardiac transplantation is planned, additional time is gained to find a donor and to relocate the family to the appropriate facility for delivery and surgery.[8, 16] In this review two patients underwent postnatal intervention. Both deliveries occurred at the institutions chosen for postnatal surgery. Prolonged survival was achieved as a result of early surgery preceded by prostaglandin infusion and monitoring of the newborn.

Prolonged emotional distress has been cited as a potential disadvantage of providing the parents with the prenatal diagnosis of hypoplastic left heart syndrome.[16] However, Veille et al.[10] suggested that full disclosure of all information obtained from prenatal studies along with prognosis and treatment options is of paramount importance in decision-making for couples with this in utero diagnosis. In addition, we believe that in utero diagnosis of this defect is ultimately beneficial to the parents. The process of obtaining truly informed consent for either postnatal treatment or nontreatment is clearly facilitated when the diagnosis is made antenatally, when there are multiple opportunities for continued discussions with care givers. The couple gains time and perspective and is able to start coping with their initial feelings of grief and denial. Decisions regarding postnatal management will then be made in a more appropriately thoughtful manner, rather than during the unexpected emotional turmoil brought on by an unexpected neonatal diagnosis. All patients in our series were counseled regarding the prognosis of infants with hypoplastic left heart syndrome at the time of prenatal diagnosis. Postnatal modes of treatment were explained, as was the option of nontreatment. Discussion of elective abortion was offered as a legal option for previable pregnancies.

The risk of misdiagnosis is certainly a potential disadvantage, as has been cited by Johnston and Sakala.[16] Fetal echocardiography has been shown to be accurate in defining fetal cardiac anatomy[17] and in the detection of congenital heart defects.[18, 19] In addition, recent advances in ultrasonographic technology have provided systems that facilitate correct in utero diagnosis of congenital heart defects. Pulsed Doppler is accepted as an important adjunct to two-dimensional fetal echocardiography, and the use of this technology to diagnose hypoplastic left heart syndrome prenatally has been reported.[20, 21] Color flow mapping facilitates identification of small cardiac and vascular structures, allowing real-time visualization of direction of blood flow in cardiac structures and great vessels.[22, 23] Chiba et al.[24] suggested that in their hands color flow mapping increased the sensitivity of fetal echocardiography to correctly diagnose fetal structural heart disease above that of conventional two-dimensional imaging (from 58% to 76%), although we have not found color flow to improve the sensitivity of structural diagnosis in our laboratory.[25] The use of this technique to diagnose hypoplastic left heart syndrome has been reported.[23-27] We found color flow Doppler to be helpful in diagnosing hypoplastic left heart syndrome in 10 of the 15 cases in which it was used. This technology combined with experience in fetal echocardiography minimizes the risk of misdiagnosis. We have not documented false-positive diagnoses of hypoplastic left heart syndrome and believe that if a skilled observer performs these studies such misdiagnosis should be virtually impossible.

The use of the four-chamber view as a sensitive screen for intracardiac malformations has been documented.[28] Fourteen of the 20 cases presented had fetal echocardiography because of an abnormal four-chamber view. We anticipate that as the use of this screening tool increases the prenatal diagnosis of congenital heart defects will improve.

In conclusion, we have reported a series of pregnancies with hypoplastic left heart syndrome diagnosed in utero by conventional fetal echocardiography supplemented by pulsed and color flow Doppler. These pregnancies exhibited poor perinatal survival in part because of associated genetic and structural abnormalities that resulted in elevated intrauterine and neonatal mortality. Prolonged survival was achieved in two cases with prenatal diagnosis and counseling and afforded the opportunity of early neonatal intervention. Neonatal management of these infants is evolving. Although the Norwood procedure[9] and neonatal transplantation[8] are innovative techniques, there are few long-term clinical data currently available. The neonatal counseling for these families is therefore complex. We have found that by obtaining diagnostic information prenatally we have been able to afford this population an opportunity to make more informed decisions than had they been compelled to decide under the pressure of the neonatal intensive care setting. It is anticipated that fetal echocardiography will continue to be an accurate tool for the in utero diagnosis of severe congenital heart defects and will contribute to the management of pregnancies so complicated and to preparations for neonatal surgical intervention.

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Adrenal and ovarian steroid hormone responses to gonadotropin-releasing hormone agonist treatment in polycystic ovary syndrome

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It has been postulated that in polycystic ovary syndrome ovarian steroids can influence adrenal steroidogenesis. To test this hypothesis, basal and dexamethasone-suppressed–corticotropin-stimulated steroid hormone responses were compared among three groups of women before, during, and after gonadotropin-releasing hormone agonist treatment for 3 months. The groups were characterized as follows: (1) women with polycystic ovary syndrome with high dehydroepiandrosterone sulfate levels (>400 μg/dl), (2) women with polycystic ovary syndrome with normal dehydroepiandrosterone sulfate levels (<300 μg/dl), and (3) normal ovulatory women. In response to gonadotropin-releasing hormone agonist, basal serum luteinizing hormone, follicle-stimulating hormone, estradiol, estrone, 17-hydroxyprogesterone, androstenedione, and testosterone in all three groups were suppressed to similar levels. Basal serum dehydroepiandrosterone sulfate levels in the group with high levels declined, but they did not reach the normal, unaltered concentrations in the other two groups. Two subjects with polycystic ovary syndrome in this group with high levels, who showed the greatest declines in basal serum dehydroepiandrosterone sulfate levels (34%, 40%), also had evidence of 3β-hydroxysteroid dehydrogenase deficiency before treatment, which was resolved by the end of treatment. In both groups with polycystic ovary syndrome, the increase in maximum incremental rise of dehydroepiandrosterone and dehydroepiandrosterone sulfate levels in response to a pharmacologic dose of corticotropin from a dexamethasone-suppressed baseline (adrenal androgen capacity) remained unaltered during gonadotropin-releasing hormone agonist administration. We conclude that ovarian steroids may promote excessive adrenal androgen secretion in women with polycystic ovary syndrome, may induce 3β-hydroxysteroid dehydrogenase deficiency as a mechanism for adrenal involvement in some women with polycystic ovary syndrome, and do not influence adrenal androgen capacity. (AM J OBSTET GYNECOL 1991;165:535-45.)

Key words: Polycystic ovary syndrome, gonadotropin-releasing hormone agonist, dehydroepiandrosterone sulfate, 3β-hydroxysteroid dehydrogenase deficiency

The mechanism of excess adrenal androgen secretion in polycystic ovary syndrome remains controversial. It has been postulated that ovarian steroids influence adrenal steroidogenesis by inhibition of adrenal 3β-hydroxysteroid dehydrogenase activity or by causing adrenal androgen hyperresponsiveness to corticotropin. This hypothesis may account for the elevations in serum levels of dehydroepiandrosterone sulfate (DHA-S) observed in approximately half of women with polycystic ovary syndrome.[1,2] Administration of a long-acting gonadotropin-releasing hormone (GnRH) agonist has been reported to selectively suppress pituitary gonadotropins and secretion of ovarian steroids in women with polycystic ovary syndrome.[3,4] However, the effect of administering GnRH agonist to women with polycystic ovary syndrome with high DHA-S levels (>400 μg/dl) has never been examined. Thus this study was undertaken to determine if women with polycystic ovary syndrome and high DHA-S levels have a different adrenal response than do women with polycystic ovary syndrome and normal DHA-S levels to GnRH agonist administration for 3 months. Basal and dexamethasone-suppressed–corticotropin-stimulated hormone profiles were evaluated before, during, and after treatment, and the results were compared with those in a group of normal ovulatory women who were similarly evaluated for the same period of time.

Material and methods

Subjects. Ten women with polycystic ovary syndrome were selected for study because they showed elevation in at least one serum androgen level (DHA-S, >340

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Table I. Baseline clinical data in 10 women with polycystic ovary syndrome

Subject No.	Age (yr)	Ideal body weight (%)	Menses (cycles/yr)	Hirsutism	Ovarian appearance	
					Enlarged*	Polycystic†
High DHA-S						
2	18	142	3	+	−	+
4	25	182	6	+	+	−
7	22	173	6	+	−	+
8	19	126	2	+	−	−
10	31	176	3	+	−	+
Normal DHA-S						
1‡	23	173	7	+	+	+
3	33	168	5	+	+	+
5	35	139	3	+	+	+
6	35	212	1	+	+	+
9	27	120	6	+	+	+

+, Positive; −, negative.
*Both ovaries >4.5 cm in length.
†Both ovaries with more than 5 follicles <10 mm in diameter.
‡Acanthosis nigricans.

μg/dl; total testosterone, >0.7 ng/ml, or free testosterone, >0.8 ng/ml) and exhibited the classic features of oligomenorrhea and hirsutism. All had normal prolactin levels (<20 μg/L), were euthyroid (thyroid-stimulating hormone, <3.5 mIU/L), and had a positive response to medroxyprogesterone withdrawal. The clinical characteristics of these women are shown in Table I. All were between 18 and 35 years of age, nine were obese (>120% of ideal body weight), and one had acanthosis nigricans. Although all subjects with polycystic ovary syndrome underwent vaginal ultrasonography for ovarian characterization before the study, enlarged or polycystic ovaries were not required for the diagnosis of polycystic ovary syndrome. In retrospect, all exhibited low-dose dexamethasone suppression of cortisol to <5 μg/dl, as well as basal serum and postcorticotropin 17-hydroxyprogesterone levels that were <3 ng/ml at the beginning of the study to exclude Cushing's disease and attenuated 21-hydroxylase deficiency, respectively.

Three groups of subjects were studied, after institutional review board approval and informed consent. Basal serum DHA-S levels drawn at the beginning of the study were used to assign half (n = 5) of the subjects with polycystic ovary syndrome to the high DHA-S group (>400 μg/dl) and the remaining half to the normal DHA-S group (<300 μg/dl). The third group consisted of five normal ovulatory women similar in age to the subjects with polycystic ovary syndrome. All had regular menses and no evidence of hirsutism. All demonstrated a biphasic basal body temperature curve and a luteal range serum progesterone level (>6 ng/ml) after the temperature shift, in the cycle before the study was begun. None of the subjects in any group had received hormonal medication for at least 6 weeks before the study.

Protocol. All three groups received a GnRH agonist (leuprolide acetate, TAP Pharmaceuticals, North Chicago; 2 mg self-injected subcutaneously daily at 8 AM) for 3 months. The injections in subjects with polycystic ovary syndrome were begun without regard to the patient's previous menstrual period and in ovulatory women during the mid to late follicular phase of the menstrual cycle.

Each subject underwent a baseline evaluation of basal secretion and adrenal dynamic response during the week before GnRH agonist treatment was started. Blood samples were collected at 8 AM every 3 days (−8, −5, and −2 days) for calculation of the mean to represent basal secretion. On the evening of the final basal blood drawing, study subjects were admitted to the General Clinical Research Center of University Hospitals of Cleveland. Dexamethasone (1 mg orally) was given at 11 PM followed by an intravenous bolus of 0.25 mg synthetic corticotropin (1-24) (Cortrosyn, Organon, West Orange, N.J.) at 8 AM the next morning. Blood samples were collected through an indwelling catheter at −15, 0, 60, and 180 minutes after corticotropin injection. During GnRH agonist administration, blood samples were drawn at monthly intervals at 8 AM just before the daily injection of the medication. Adrenal dynamic testing was repeated after the first month and at the end of treatment, but on these occasions, the GnRH agonist injection was given at 7:30 AM, 30 minutes before the corticotropin injection.

The serum levels used for screening patients were measured in the General Chemistry Laboratory of University Hospitals of Cleveland from blood samples drawn within 6 months before the study in women with polycystic ovary syndrome and during the cycle before the study in ovulatory women. Screening levels of DHA-S, total testosterone, and progesterone were mea-

Table II. Baseline hormone levels (mean ± SD) in patients from all three groups

Hormone	*High DHA-S*	*Normal DHA-S*	*Ovulatory*
LH (mIU/ml, IU/L)	21.3 ± 7.9, 21.3 ± 7.9*	14.1 ± 8.1, 14.1 ± 8.1	8.3 ± 10.2, 8.3 ± 10
FSH (mIU/ml, IU/L)	10.9 ± 3.5, 10.9 ± 3.5	8.4 ± 1.5, 8.4 ± 1.5	12.8 ± 15.7, 12.8 ± 15
Estrone (pg/ml, pmol/L)	59.7 ± 24.6, 220.0 ± 91.0	74.3 ± 43.3, 274.8 ± 160.2	44.8 ± 9.3, 165.7 ± 37
Estradiol (pg/ml, pmol/L)	82.2 ± 84.7, 301.8 ± 310.9	59.2 ± 28.5, 217.3 ± 104.6	71.7 ± 49.9, 263.2 ± 18
17-Hydroxypregnenolone (ng/ml, nmol/L)	4.6 ± 3.3, 13.8 ± 9.9	2.1 ± 1.3, 6.3 ± 3.9	1.8 ± 1.1, 5.4 ± 3.3
17-Hydroxyprogesterone (ng/ml, nmol/L)	1.4 ± 0.7, 4.2 ± 2.1	1.0 ± 0.5, 30.0 ± 1.5	1.0 ± 1.0, 3.0 ± 3.0
Androstendione (ng/ml, nmol/L)	4.1 ± 1.0, 14.3 ± 3.5†‡	3.4 ± 1.3, 11.9 ± 4.5	2.7 ± 0.5, 9.4 ± 1.7
Testosterone (ng/ml, nmol/L)	0.7 ± 0.2, 2.4 ± 0.7†‡	0.9 ± 0.6, 3.1 ± 2.1§	0.4 ± 0.1, 1.4 ± 0.3
DHA (ng/ml, nmol/L)	7.7 ± 2.9, 26.7 ± 10.0†	4.3 ± 1.3, 14.9 ± 4.5	4.4 ± 1.3, 15.3 ± 4.5
DHA-S (μg/dl, μmol/L)	453.0 ± 43.3, 12.3 ± 1.2†	210.0 ± 74.0, 5.7 ± 1.9	175.0 ± 36.9, 4.7 ± 1.0
Cortisol (μg/dl, nmol/L)	20.6 ± 5.3, 568.4 ± 146.2	15.5 ± 3.1, 427.6 ± 85.5	18.2 ± 4.9, 507.0 ± 35

*$p = 0.06$, high DHA-S group compared with ovulatory group.

†$p < 0.05$, high DHA-S group compared with ovulatory group.

‡$p < 0.05$, high DHA-S group compared with normal DHA-S group.

§$p = 0.08$, normal DHA-S group compared with ovulatory group.

sured with radioimmunoassay kits obtained from Pantex Corp. (Santa Monica, Calif.), while those for prolactin and thyroid-stimulating hormone were measured with radioimmunoassay kits obtained from DELFIA, Pharmacia ENI, Inc. (Fairfield, N.J.). Screening levels of free testosterone were measured by a method described previously.[5] Actual study data were generated by measuring serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, androstenedione, testosterone, 17-hydroxyprogesterone, dehydroepiandrosterone (DHA), DHA-S, and cortisol with radioimmunoassay kits obtained from Diagnostic Products Corp. (Los Angeles) or by Nichols Institute (San Juan Capistrano, Calif.), where serum estrone and 17-hydroxypregnenolone were measured. For a given hormone all samples from an individual patient were measured in duplicate in the same assay. The intraassay and interassay coefficients of variation were below 9% and 12.3%, respectively, for all assays, except those for the 17-hydroxypregnenolone assay, which were 9.3% and 15.5%, respectively.

The adrenal response after corticotropin injection during baseline dynamic testing (0 months) was used to determine adrenal enzyme activities, adrenal androgen capacity, and cortisol output. The mean ratios of the maximum absolute values of the appropriate substrates to products in the high and normal DHA-S groups were compared with those in ovulatory women to detect differences in the activities of 3β-hydroxysteroid dehydrogenase and 17,20-lyase in either group with polycystic ovary syndrome. With criteria similar to those described previously,[6] women with polycystic ovary syndrome in whom the maximum absolute values of DHA or 17-hydroxypregnenolone and the ratios of the maximum absolute values of DHA to androstenedione and 17-hydroxypregnenolone to cortisol were >2 SD above the mean of ovulatory women were considered to have 3β-hydroxysteroid dehydrogenase deficiency. Ratios of the maximum absolute values of 17-hydroxypregnenolone to DHA or 17-hydroxyprogesterone to androstenedione that were >2 SD below the mean of normal ovulatory women were required to fulfill the criteria for 17,20-lyase hyperactivity. Cortisol output was defined as the maximum absolute value of cortisol secreted. The maximum incremental rise in DHA and DHA-S secretion from a standardized dexamethasone-suppressed baseline (mean of values at −15 and 0 minutes) was calculated to represent adrenal androgen capacity. Alterations in these parameters during GnRH agonist administration were determined from adrenal dynamic testing performed after 1 month and 3 months of treatment.

Statistical evaluation was performed with two-way analysis of variance, followed by Student paired or group *t* tests, where appropriate. One-tailed significance was considered sufficient when paired comparisons were assessed for the basal serum levels of LH, FSH, estradiol, estrone, 17-hydroxyprogesterone, androstenedione, and testosterone because only declines in these hormones were anticipated in response to GnRH agonist, as demonstrated previously.[3,4,7,8] However, two-tailed significance was used for all other comparisons. The Bonferroni test was chosen to control for multiple comparisons when differences across groups during treatment were assessed.[9] In this instance a value of 0.017 was used to detect a significant difference at the level of 0.05. Linear regression was used to examine correlations in the responses to GnRH agonist among the basal serum levels of estradiol, estrone, and DHA-S.

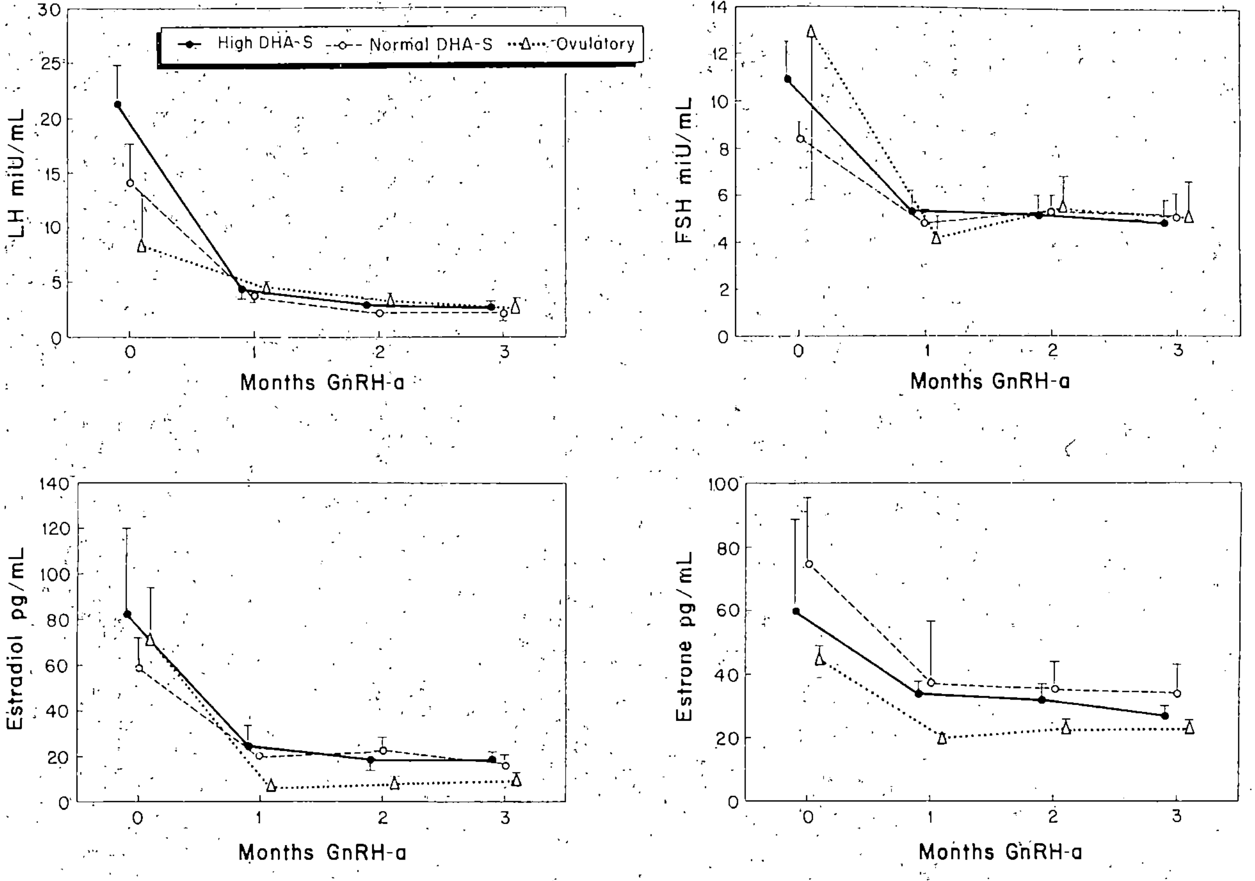

Fig. 1. Basal serum LH, FSH, estradiol, and estrone concentrations (mean ± SE) in women with polycystic ovary syndrome with high and normal DHA-S levels and in ovulatory women before and during GnRH agonist *(GnRH-a)* treatment. Factors used to convert data into Systèm International d'Unités (SI units) are 3.671 and 3.699 for estradiol and estrone, respectively.

Results

Basal hormonal response

Baseline hormone concentrations. The baseline hormonal characteristics of the three treatment groups are shown in Table II. The mean serum concentrations of androstenedione, testosterone, DHA, and DHA-S just before treatment were significantly higher ($p < 0.05$) in the high DHA-S group than in ovulatory women. Baseline hormonal differences between the polycystic ovary syndrome groups were limited to those in mean serum DHA and DHA-S levels, which were significantly higher ($p < 0.05$) in the high DHA-S group than in the normal DHA-S group. Both polycystic ovary syndrome groups demonstrated elevations in serum androgens and elevated LH to FSH ratios of 2.0 and 1.7 in the high DHA-S group and the normal DHA-S group, respectively. A reversal of the normal estradiol to estrone ratio also was evident in the normal DHA-S group.

Gonadotropins and estrogens. As shown in Fig. 1, basal gonadotropin levels (mean ± SE) before and during GnRH agonist administration in the high and normal DHA-S groups and ovulatory women are compared with each other. Administration of GnRH agonist for 1 month resulted in marked and significant reductions ($p < 0.05$) of mean serum LH and FSH levels in both groups with polycystic ovary syndrome. These reductions were maintained throughout the entire treatment period. In ovulatory women, gonadotropin concentrations exhibited only modest declines. However, respective serum levels of LH and FSH were similar in all three groups throughout the course of GnRH agonist therapy.

The serum estradiol and estrone responses to GnRH agonist after 1 month of treatment (Fig. 1) also were characterized by marked and significant reductions ($p < 0.05$) in all three groups. Respective serum levels of estradiol and estrone remained low and were similar in all three groups throughout the treatment period. In the case of serum estradiol, levels during treatment approached the limits of sensitivity (10 pg/ml or 36 pmol/L) for the assay.

Progestins. The basal serum progestin levels (mean ± SE) before and during GnRH agonist treatment are shown in Fig. 2. Serum 17-hydroxypregnen-

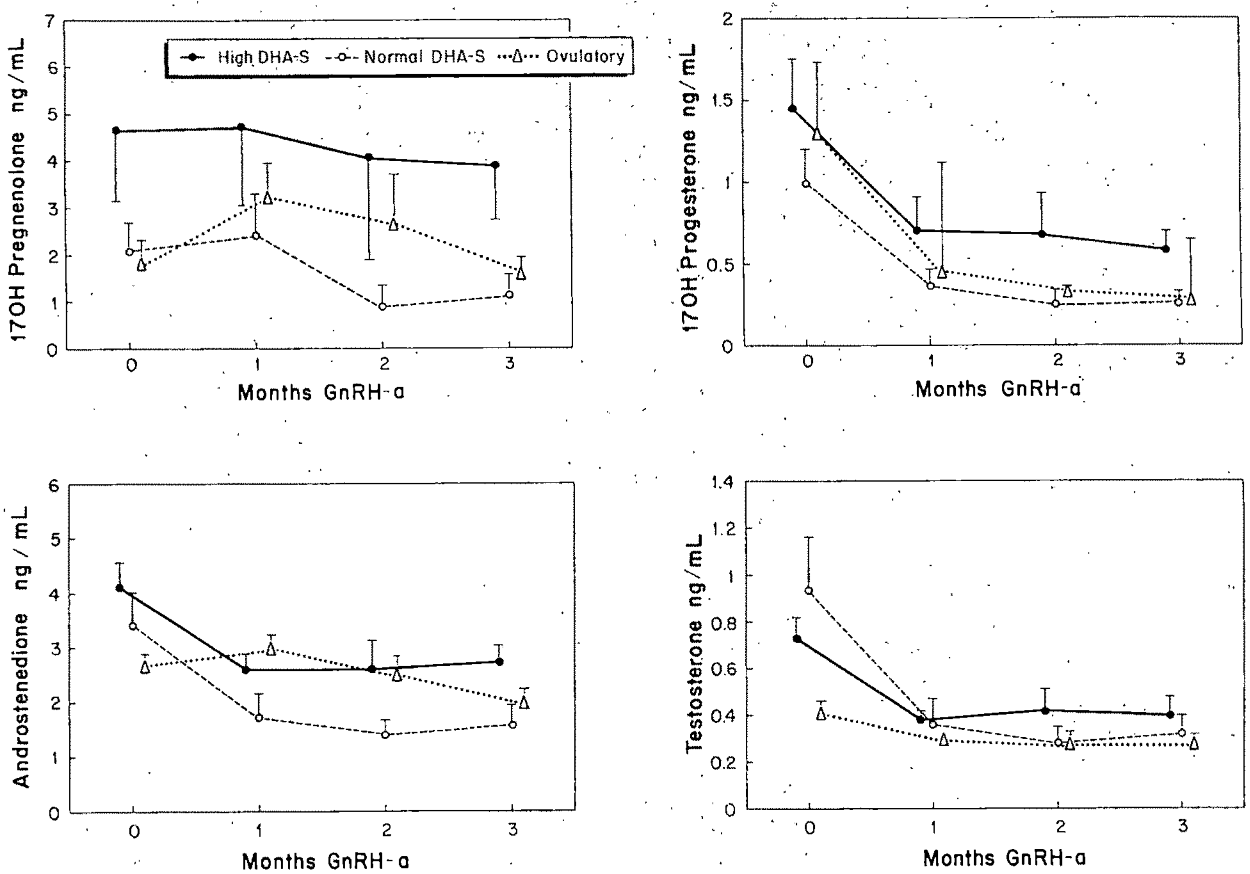

Fig. 2. Basal serum 17-hydroxypregnenolone, 17-hydroxyprogesterone, androstenedione, and testosterone concentrations (mean ± SE) in women with polycystic ovary syndrome with high and normal DHA-S levels and in ovulatory women before and during GnRH agonist *(GnRH-a)* treatment. Factors used to convert data into SI units are 3.003 (17-hydroxypregnenolone), 3.026 (17-hydroxyprogesterone), 3.492 (androstenedione), and 3.467 (testosterone).

olone measurements during treatment never differed significantly from baseline levels in all three groups. In contrast, the considerable reductions in serum 17-hydroxyprogesterone levels that occurred after 1 month of GnRH agonist administration were significant ($p < 0.05$) in both groups with polycystic ovary syndrome. Low serum levels of 17-hydroxyprogesterone persisted in all three groups for the remainder of the study.

Androgens and cortisol. Also depicted in Fig. 2 are the mean basal serum levels of androstenedione and testosterone before and during GnRH agonist administration. A significant reduction in mean serum androstenedione concentrations occurred in the high DHA-S group by 37% ($p = 0.01$) and in the normal DHA-S group by 50% ($p = 0.046$) after the first month of treatment. These low levels were maintained throughout the remainder of the study. In contrast, a modest decline of 23% in the mean serum androstenedione level in ovulatory women was observed only after 3 months of GnRH agonist therapy, which closely approached significance ($p = 0.05$). Serum testosterone levels declined promptly in all three groups. After 1 month of GnRH agonist treatment, the mean serum testosterone levels had fallen significantly by 48% in the high DHA-S group ($p = 0.005$) and by 61% in the normal DHA-S group ($p = 0.015$). Serum testosterone levels remained low and were similar among the three groups for the remainder of the treatment period.

As shown in Fig. 3, the serum concentrations (mean ± SE) of DHA in the high DHA-S group and in ovulatory women, as well as that of DHA-S in the normal DHA-S group and in ovulatory women, remained unaltered throughout GnRH agonist administration. In the normal DHA-S group, the serum DHA levels remained similar to those in ovulatory women during the entire course of GnRH agonist therapy. In the high DHA-S group, the mean serum DHA-S level underwent a progressive linear decline of 24%, to 343.4 ± 39.0 μg/dl (9.1 ± 1.1 μmol/L), which achieved significance ($p = 0.012$) after the third month of GnRH agonist administration. Comparisons between

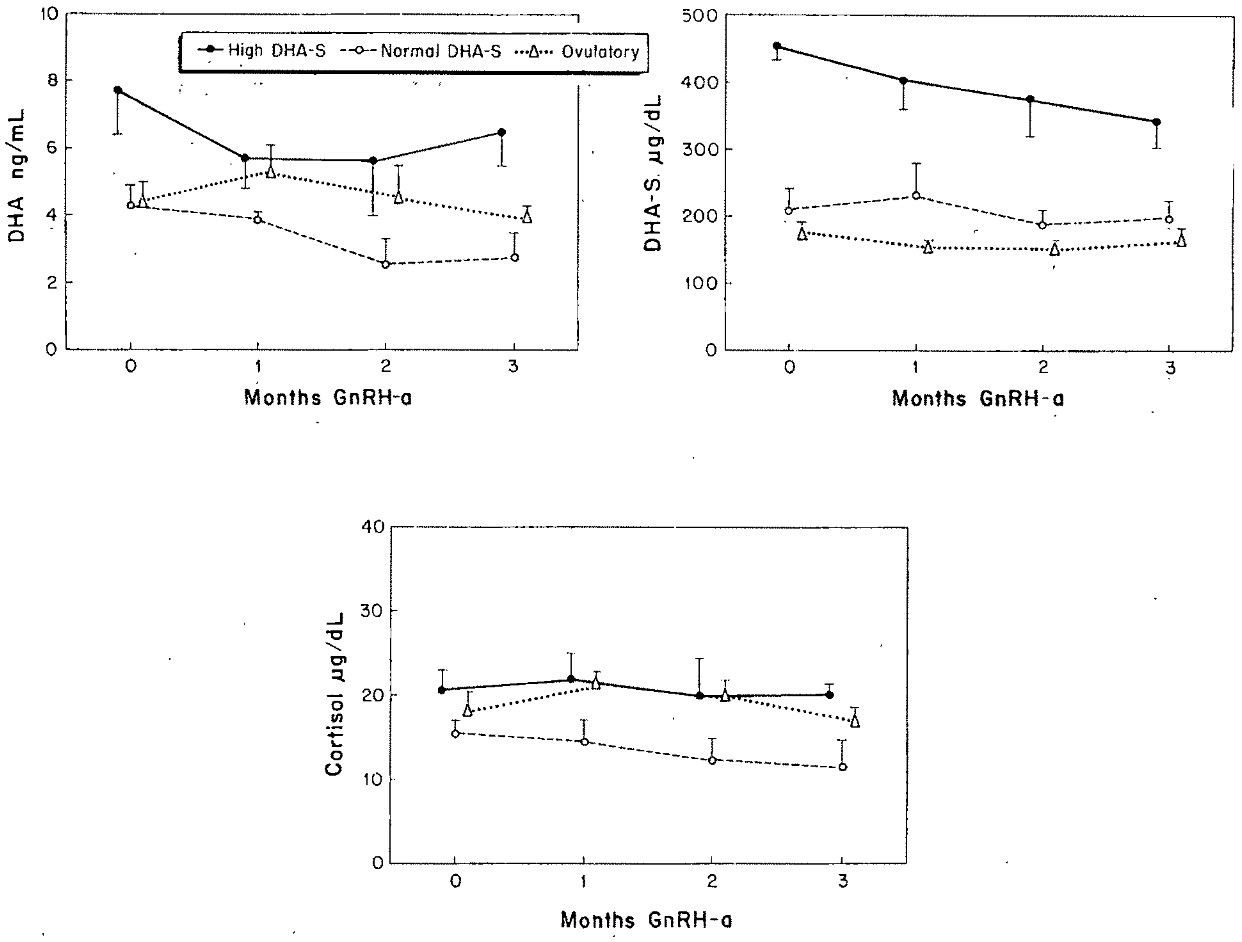

Fig. 3. Basal serum DHA, DHA-S, and cortisol concentrations (mean ± SE) in women with polycystic ovary syndrome with high and normal DHA-S levels and in ovulatory women before and during GnRH agonist *(GnRH-a)* treatment. Factors used to convert data into SI units are 3.467 (DHA), 0.02714 (DHA-S), and 27.59 (cortisol).

this reduction in serum DHA-S with those of either serum estradiol or estrone in the high DHA-S group with the use of regression and correlation yielded correlation coefficients of 0.13 and 0.33, respectively. In contrast, a similar analysis comparing the decline in these estrogens with each other yielded a much higher correlation coefficient of 0.85. The mean DHA-S concentration in the high DHA-S group did not achieve similarity to that of the normal DHA-S group or ovulatory women by the end of the study. Finally, serum cortisol measurements did not change in any of the three groups throughout the course of treatment.

Adrenal dynamic response. The maximal postcorticotropin responses for DHA, androstenedione, 17-hydroxypregnenolone, 17-hydroxyprogesterone, and cortisol were achieved at 60 minutes, then declined significantly or had sustained values at 180 minutes. In contrast, the maximal postcorticotropin response for DHA-S was not achieved until 180 minutes. Similar responses for these steroids have been reported previously.[10] The ratios of 17-hydroxypregnenolone to cortisol, DHA to androstenedione, and 17-hydroxypregnenolone to 17-hydroxyprogesterone and cortisol output were calculated at 60 minutes. The maximum incremental rises of DHA and DHA-S were calculated at 60 and at 180 minutes, respectively.

Adrenal enzyme activities. The postcorticotropin ratios (mean ± SE) of DHA to androstenedione, 17-hydroxypregnenolone to 17-hydroxyprogesterone, and 17-hydroxypregnenolone to cortisol in all three groups before, during, and after GnRH agonist administration are shown in Fig. 4. Before treatment, the mean ratios of all three of these Δ^5- to Δ^4-steroid pairs were similar in all three groups. However, individual analysis of women with polycystic ovary syndrome suggests altered 3β-hydroxysteroid dehydrogenase activity in two subjects (No. 4 and No. 10) in the high DHA-S group depicted by triangles in Figs. 4 and 5, who showed the greatest decline in basal secretion of DHA-S (34% and 40%, respectively) during treatment. Before GnRH agonist administration, subject 10 met all the criteria for 3β-hydroxysteroid dehydrogenase de-

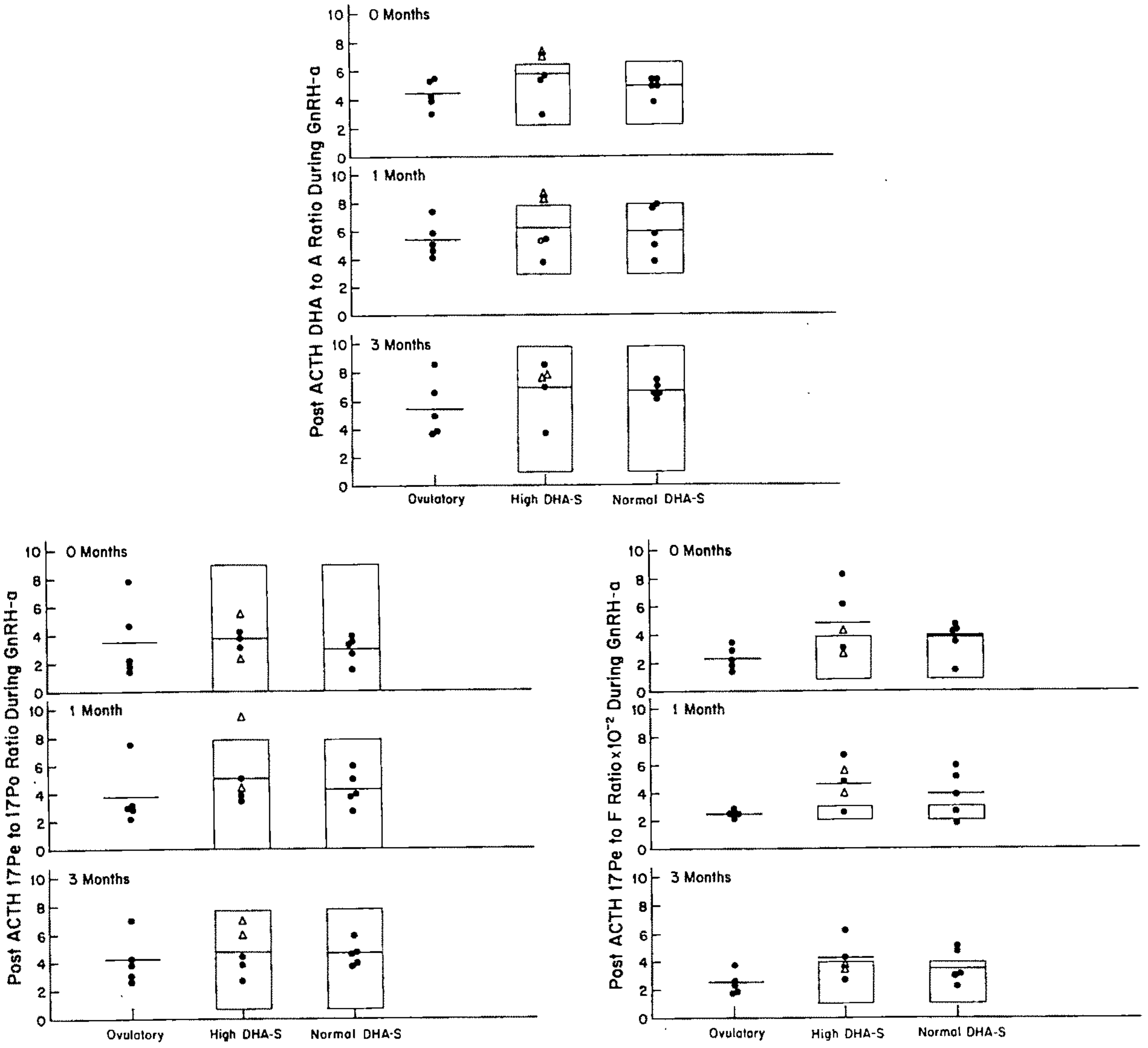

Fig. 4. Postcorticotropin ratios of DHA to androstenedione *(A)*, 17-hydroxypregnenolone *(17Pe)* to 17-hydroxyprogesterone *(17Po)*, and 17-hydroxypregnenolone to cortisol *(F)* for women with polycystic ovary syndrome with high and normal DHA-S levels and in ovulatory women at 0, 1, and 3 months of GnRH agonist *(GnRH-a)* treatment. Two women with high levels of DHA-S (△) met the criteria for 3β-hydroxysteroid dehydrogenase deficiency at 0 or 1 month or both of these intervals.

ficiency whereas subject 4 met only two of three criteria. After 1 month of GnRH agonist treatment, both of these subjects showed all the criteria for 3β-hydroxysteroid dehydrogenase deficiency. End-of-treatment values in both subjects revealed postcorticotropin levels of DHA and 17-hydroxypregnenolone that remained similar to the 1-month treatment values but postcorticotropin ratios of DHA to androstenedione and 17-hydroxypregnenolone to cortisol that were <2 SD above the mean of ovulatory women. These latter findings suggest a resolution of the 3β-hydroxysteroid dehydrogenase deficiency in response to GnRH agonist therapy.

None of the women with polycystic ovary syndrome from either the high or normal DHA-S group met the criteria for 17,20-lyase hyperactivity during baseline dynamic testing. There was also no evidence of altered 17,20-lyase activity in women with polycystic ovary syndrome either before or in response to GnRH agonist therapy when the mean substrate to product ratios for this enzyme were compared among all three groups.

Cortisol output. As shown in Fig. 5, cortisol output (mean ± SE) in the high DHA-S group (27.3 ± 0.9 μg/dl or 753.2 ± 24.8 nmol/L), in the normal DHA-S group (29.8 ± 1.9 μg/dl or 822.2 ± 57.4 nmol/L) and in ovulatory women (25.3 ± 30.0 μg/dl or 698.0 ± 82.8 nmol/L) were not significantly different before GnRH agonist administration and remained similar af-

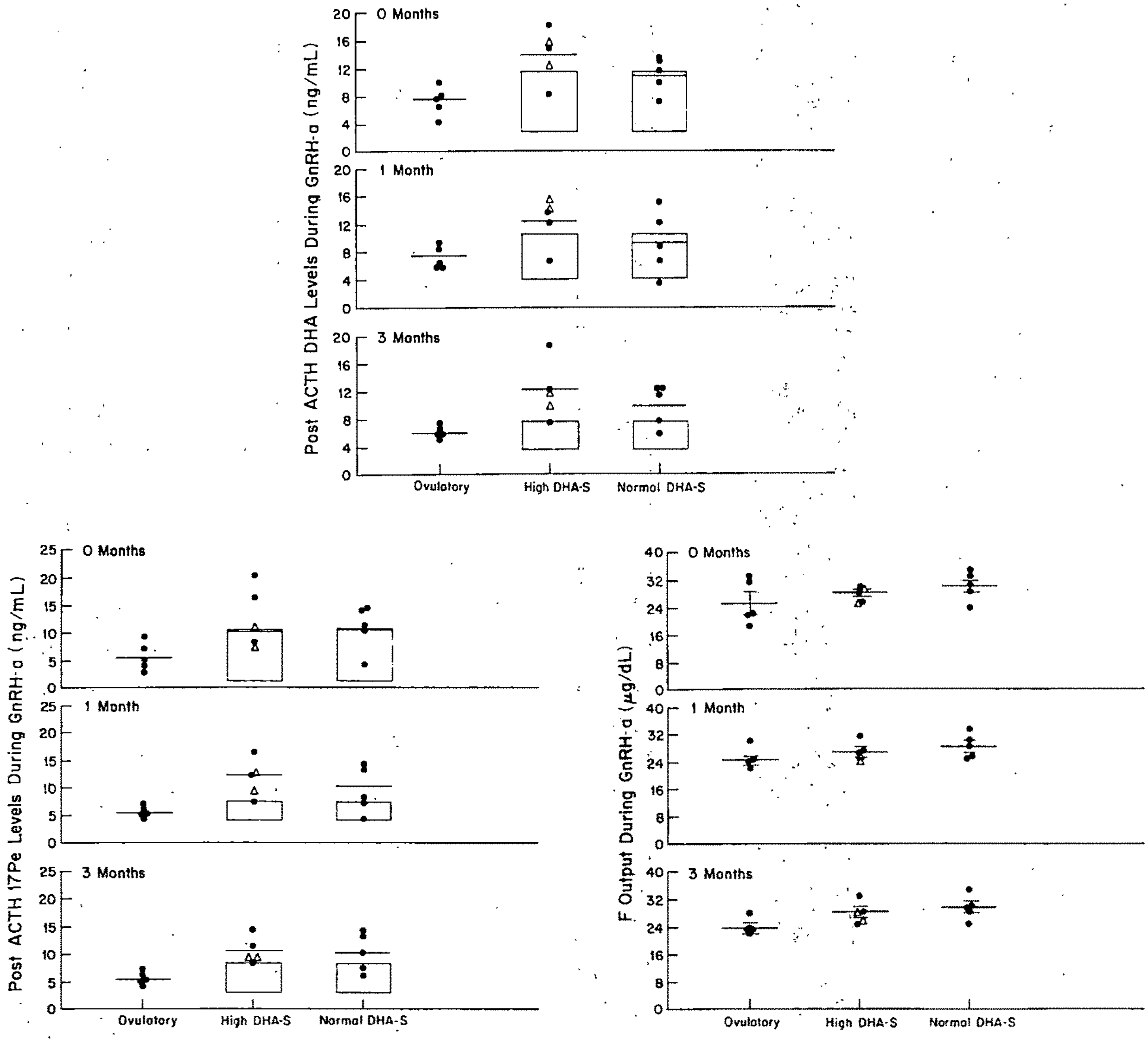

Fig. 5. Postcorticotropin levels of DHA and 17-hydroxypregnenolone *(17Pe)*, as well as cortisol *(F)* output, in women with polycystic ovary syndrome with high and normal DHA-S levels and in ovulatory women at 0, 1, and 3 months of GnRH agonist *(GnRH-a)* treatment. Two women with high DHA-S levels (Δ) met criteria for 3β-hydroxysteroid dehydrogenase deficiency at 0 or 1 month or both of these intervals.

ter 1 month of treatment. By the end of the study cortisol output in the high DHA-S group (28.6 ± 1.1 µg/dl or 789.1 ± 30.3 nmol/L) was again similar to that in the normal DHA-S group (29.4 ± 1.6 µg/dl or 800.1 ± 44.1 nmol/L) but was significantly greater ($p = 0.014$) by 19% compared with that in ovulatory women (24.0 ± 1.0 µg/dl or 662.2 ± 27.6 nmol/L).

Adrenal androgen capacity. Measurements of the maximum incremental rise (mean ± SE) representing the adrenal capacity for DHA and DHA-S before, during, and after GnRH agonist treatment are shown in Fig. 6. The mean baseline maximum incremental rises of both adrenal androgens were greater in the high DHA-S group than in the normal DHA-S group and greater in the normal DHA-S group than in ovulatory women. Throughout the course of GnRH agonist administration, the difference in maximum incremental rises of either adrenal androgen between the high and normal DHA-S group did not achieve significance. However, the maximum incremental rises of both adrenal androgens were significantly greater ($p < 0.05$) in the high DHA-S group than in ovulatory women after 1 month and 3 months of GnRH agonist therapy. In spite of the differences between either group with polycystic ovary syndrome and ovulatory women by the end of the study, the maximum incremental rises of DHA and DHA-S remained unaltered in both groups with polycystic ovary syndrome compared with respective baselines throughout the entire treatment period. Therefore the pattern of adrenal capacity ob-

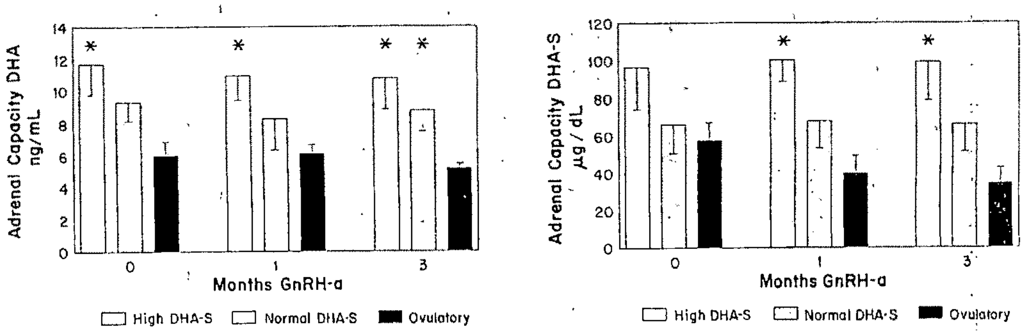

Fig. 6. Measurements of maximum incremental rise (mean ± SE) representing adrenal capacity for DHA and DHA-S in women with polycystic ovary syndrome with high and normal DHA-S levels and in ovulatory women at 0, 1, and 3 months of GnRH agonist *(GnRH-a)* treatment. *Asterisk,* Significant difference ($p < 0.05$) between either group of women with polycystic ovary syndrome and ovulatory women.

served during baseline dynamic testing (the high DHA-S group > the normal DHA-S group > ovulatory women) persisted in both adrenal androgens on completion of GnRH agonist therapy.

Comment

The baseline hormonal concentrations in women with polycystic ovary syndrome were similar to previously reported results for this syndrome.[11] In both groups with polycystic ovary syndrome, complete suppression of ovarian steroidogenesis was apparent after 1 month of GnRH agonist treatment. This is evident by the reductions in respective basal serum concentrations of estradiol, estrone, 17-hydroxyprogesterone, androstenedione, and testosterone to similar levels during this time. Whereas basal serum estradiol levels were nearly undetectable, the detectable levels of basal serum estrone, 17-hydroxyprogesterone, androstenedione, and testosterone were most likely derived from the adrenal glands, either by direct secretion or by peripheral conversion of an adrenal precursor. The persistence of this phenomenon for the entire 3 months of therapy is consistent with a previous report[4] indicating that prolonged GnRH agonist administration induces and maintains declines in ovarian steroids in the castrate range.

In all three groups it is likely that the sustained declines in basal serum FSH during therapy caused the corresponding decreases in basal serum estradiol and estrone. In addition, the sustained declines in basal serum LH throughout GnRH agonist treatment coincided with declines in basal serum androstenedione, testosterone, and 17-hydroxyprogesterone. Results from Rittmaster[7] suggest that optimal suppression of basal serum LH with a dose of leuprolide acetate similar to that used in our study is required for maximum reductions of ovarian testosterone concentrations in women with polycystic ovary syndrome, even though serum estradiol levels can be easily suppressed on half of the GnRH agonist dose selected. In fact, the mean concentrations of testosterone observed during treatment in the women with polycystic ovary syndrome in our study (48% and 61% for the high and normal DHA-S groups, respectively) were consistent with the maximal GnRH agonist suppressed levels (56%) previously described for this syndrome.[7]

It is unlikely that GnRH agonist administration is capable of directly affecting adrenal steroidogenesis. Basal serum cortisol, the major secretory product of the adrenal, remained unaltered within the normal range in all three groups for the entire 3 months of treatment. In addition, the basal serum concentrations of DHA and DHA-S, androgens of predominantly adrenal origin, remained within the normal range in the normal DHA-S group and in ovulatory women throughout the course of the study. These findings are well documented by previous investigators.[3, 4, 8] Moreover, the responses of these adrenal androgens in the normal DHA-S group and in ovulatory women to GnRH agonist–induced ovarian suppression suggest that ovarian steroids are not responsible for the maintenance of normal adrenal androgen secretion in reproductive-age women, regardless of whether they are ovulatory or have polycystic ovary syndrome.

In contrast, ovarian steroids appear to be capable of promoting excess adrenal androgen secretion. This is evident in the high DHA-S group by the gradual but significant decline in basal serum DHA-S toward the normal range in response to 3 months of GnRH agonist administration. This pattern of response in serum DHA-S is unlikely to be ovarian, given its poor correlation with the immediate sharp decline in ovarian estrogens and the lack of significant change in its immediate precursor, DHA in the high DHA-S group. It is well established that DHA-S is quantitatively the most important adrenal androgen, given its high serum concentrations, its long half-life, and its minimal diurnal or episodic variation.[12] Consequently, the significant elevation in the pretreatment levels of basal serum DHA-S in the high DHA-S group is the most sensitive indicator of adrenal involvement in polycystic ovary syndrome,[1, 13] and the change in basal serum DHA-S in this group during GnRH agonist therapy is more likely than that of DHA to be indicative of alteration in adrenal function.

Three months of GnRH agonist administration is insufficient to correct adrenal dysfunction in the high DHA-S group. This is evident by the lack of similarity between the end-of-treatment serum DHA-S level in the high DHA-S group and the normal unaltered DHA-S concentrations in the other two groups. Although the GnRH agonist treatment length was chosen arbitrarily, consideration was given to select a reasonable period of time for sufficient adrenal morphologic alterations to occur that would cause an appropriate change in adrenal steroidogenesis.[14] However, further studies are required to determine the appropriate length of GnRH agonist administration for normalization or stabilization of basal DHA-S levels in the high DHA-S group.

It is possible that changes in adrenocortical steroidogenic enzyme activities in response to GnRH agonist treatment resulted in the normalizing trend in basal serum DHA-S secretion in the high DHA-S group. Our data suggest that some, but not all, subjects in the high DHA-S group demonstrated alteration in 3β-hydroxysteroid dehydrogenase activity. Dynamic testing before and after 1 month of GnRH agonist treatment revealed evidence of 3β-hydroxysteroid dehydrogenase deficiency in two subjects in the high DHA-S group during an interval that involved the complete elimination of ovarian steroids from the circulation.[15] After 3 months of treatment, however, normalization of 3β-hydroxysteroid dehydrogenase activity could be demonstrated in each of these individuals. 3β-Hydroxysteroid dehydrogenase is a key branch point enzyme whose activity must remain low for adrenal androgen synthesis to be favored.[16] It is possible that ovarian steroids exert an extrinsic inhibitory influence on 3β-hydroxysteroid dehydrogenase activity leading to the elevations in serum DHA-S levels in some subjects in the high DHA-S group. However, the exact mechanism by which ovarian steroids could cause this inhibition and the possible role of other factors such as insulin or insulin-like growth factors is beyond the scope of this study. Other factors must be involved since the remaining women with polycystic ovary syndrome in the high DHA-S group did not have 3β-hydroxysteroid dehydrogenase deficiency and because further declines in serum DHA-S concentrations were not yet achieved in spite of normalization of 3β-hydroxysteroid dehydrogenase activity in the individuals affected. Nevertheless, these findings, along with evidence of normal or better glucocorticoid performance (cortisol output) in the high DHA-S group, support the contention that mild 3β-hydroxysteroid dehydrogenase deficiency can be acquired and is not always a primary genetic defect. In fact, a genetic marker for this enzyme abnormality has yet to be identified.

Examination of adrenal androgen capacity did not indicate that ovarian steroids induce adrenal androgen hyperresponsiveness to corticotropin as a cause for adrenal involvement in polycystic ovary syndrome. First of all, the greater adrenal androgen capacity in the high DHA-S group as compared with ovulatory women during baseline testing was not a phenomenon unique to this group, because baseline adrenal androgen capacity in the normal DHA-S group also was markedly increased and only slightly lower than that in the high DHA-S group. These findings confirm those in previous studies that separately reported increased adrenal capacity for DHA in women with polycystic ovary syndrome similar to those in the normal DHA-S group[17] and for DHA-S in women with polycystic ovary syndrome similar to those in the high DHA-S group.[1] In addition, adrenal androgen capacity remained unaltered in both groups with polycystic ovary syndrome throughout the course of GnRH agonist administration. Determination of adrenal androgen capacity requires the use of pharmacologic doses of corticotropin; therefore it is still possible for the adrenal androgen response to normal levels of circulating corticotropin present in all women with polycystic ovary syndrome[1, 18] to be different in each of the two groups. In fact, women with polycystic ovary syndrome similar to those in the normal DHA-S group have a normal adrenal androgen response to physiologic doses of corticotropin from a dexamethasone-suppressed baseline (adrenal sensitivity).[17] Therefore adrenal androgen capacity does not reflect the physiologic circumstance, and the lack of change in this parameter in women with polycystic ovary syndrome during GnRH agonist therapy only indicates that the pharmacologic response to corticotropin is independent of ovarian steroid influence.

In summary, suppression of ovarian steroids during GnRH agonist administration for 3 months resulted in a decline in basal serum DHA-S in women with polycystic ovary syndrome who have adrenal involvement. This alteration in serum DHA-S, the most sensitive marker of adrenal androgen production, suggests that ovarian steroids are capable of promoting excess adrenal androgen secretion. However, a GnRH agonist treatment length of 3 months was insufficient for serum DHA-S levels to fall to the normal range in this group. Adrenal 3β-hydroxysteroid dehydrogenase deficiency may be induced by ovarian steroids to cause elevations in basal serum DHA-S in some women with polycystic ovary syndrome who have adrenal involvement, but the exact mechanism for this phenomenon remains to be elucidated. In polycystic ovary syndrome, ovarian steroids have no impact on adrenal androgen capacity. However, the possibility cannot be excluded that women with polycystic ovary syndrome who have adrenal involvement also have increased adrenal androgen sensitivity that may or may not be under the influence of ovarian steroids.

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Management of fetal hemolytic disease by cordocentesis

I. Prediction of fetal anemia

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Between January 1985 and November 1990, 128 pregnancies complicated by maternal red blood cell alloimmunization were referred to our Fetal Diagnosis and Treatment Unit. We examined the premise that an evaluation of fetal blood would accurately identify fetuses at risk of requiring antenatal transfusion therapy. Two hundred seventy-two diagnostic cordocenteses were performed. Criteria for the timing of repeat cordocenteses were developed retrospectively on the basis of the fetal hematocrit values, reticulocyte counts, and direct Coombs' test results of the first 84 pregnancies. These criteria were tested and confirmed prospectively on the next 44 pregnancies. On the basis of the first blood sample, four hematologic patterns (and their distributions) were identified in the 98 antigen-positive fetuses. *Pattern 1:* fetuses at low risk of having significant antenatal anemia (hematocrit <30%) (n = 11, 11%). These fetuses had normal hematocrit values and reticulocyte counts coupled with negative or trace-positive direct Coombs' test. No fetus in this group had significant antenatal anemia. *Pattern 2:* fetuses at intermediate risk of having anemia (n = 29, 31%). *Pattern 2 fetuses* had normal hematocrit values and either direct Coombs' titers of more than trace ≤2+ and normal reticulocyte counts or low reticulocyte counts (<2.5th percentile for gestation). Twenty-one percent (n = 6) of fetuses in pattern 2 had significant antenatal anemia. *Patterns 3 and 4:* fetuses at greatest risk of having severe anemia. These fetuses had normal hematocrit values associated with either reticulocyte counts >97.5th percentile for gestation or a direct Coombs' test ≥3+ (pattern 3, n = 49, 50%) or both, or a mild anemia (>30% but <2.5th percentile for gestation) (pattern 4, n = 9, 10%). Eighty percent (n = 39) of fetuses with pattern 3 and 90% (n = 8) with pattern 4 developed a hematocrit value <30%. We conclude that evaluation of fetal hemolytic disease with a fetal blood specimen permits the identification of fetuses at high risk of having antenatal anemia. (AM J OBSTET GYNECOL 1991;165:546-53.)

Key words: Fetal hemolytic disease, cordocentesis, fetal intravascular transfusion, immune hydrops fetalis, neonatal hyperbilirubinemia

Fetal hemolytic disease caused by maternal alloimmunization remains a significant clinical problem. Anemia is the primary fetal pathophysiologic event that may lead to edema (anasarca), fetal cardiac failure, and death. The diagnosis and treatment of fetal hemolytic disease has changed little since the landmark work of Liley and others in the late 1950s and the 1960s.[1-5] These authors developed a diagnostic and therapeutic protocol that indirectly assessed fetal hemoglobin concentration on the basis of the measurement of amniotic fluid bilirubin levels as reflected by the change in optical density measurements of 450 nm (ΔOD_{450}).[1, 3] In fetuses with evidence of significant hemolysis, fetal intraperitoneal transfusion was administered. Although intrauterine intravascular transfusion has supplanted intraperitoneal transfusion in some centers,[6-9] serial measurement of the amniotic fluid ΔOD_{450} to identify candidates for fetal blood sampling remains the diagnostic mainstay, and affected fetuses (treated or untreated) are routinely delivered at <37 weeks.[4, 5, 10, 11]

A direct evaluation of the fetus would be preferable to evaluation of amniotic fluid for a number of reasons. First, the ΔOD_{450} is an indirect reflection of hematocrit value, and there is a wide range in the fetal hematocrit value for a given ΔOD_{450} at a gestational age.[3, 12, 13] Though sensitive, amniotic fluid studies are not specific. The ΔOD_{450} can be altered by contamination with blood, other fetal diseases (e.g., congenital hepatitis), and maternal diseases (e.g., sickle cell anemia). Second,

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the initial studies of anti-D hemolytic disease that formed the basis for the Liley curve were begun at 28 weeks' gestation. It is now possible to treat fetuses with severe anemia as early as 18 weeks. Predictions of disease severity on the basis of amniotic fluid nomograms extrapolated back to the midsecond trimester are less accurate than predictions made in the third trimester, because the normal ΔOD_{450} in the second trimester is zone II rather than zone I of the Liley curve.[12] Third, the ΔOD_{450} system requires at least two amniocenteses to ascertain a pattern. Fourth, the amniotic fluid method was developed according to anti-D hemolytic disease, and the results may differ with hemolytic disease caused by other antibodies.[14, 15]

In 1985 we began to examine the premise that evaluation of fetal blood rather than amniotic fluid would more accurately predict the severity of fetal disease and the need for transfusion. Because we and others had already observed a poor relationship between the fetal hematocrit value and the ΔOD_{450},[12, 13] our goal was to establish a management scheme for red blood cell alloimmunization based on fetal blood testing. We speculated that such direct testing would minimize the number of invasive procedures and prevent unnecessary preterm delivery.

Material and methods

The University of Iowa Hospital and Clinic is the tertiary care unit for the state of Iowa. There are well-established lines of referral to the university for the evaluation of maternal alloimmunization. In our laboratory, a maternal indirect Coombs' titer ≥8 has traditionally been used as an indication for amniocentesis because severe neonatal hemolytic disease has occurred at 8 but not below. Cordocentesis was offered when the maternal indirect Coombs' test revealed an immunoglobulin G titer ≥8 and either (1) ΔOD_{450} in zone II or higher before referral, (2) known or suspected paternal heterozygosity, or (3) history of a prior, significantly affected fetus or neonate. After documenting the safety of cordocentesis,[16] we offered cordocentesis for the same indications as amniocentesis.

We sought to perform the first procedure between 24 and 26 weeks unless the clinical history or ultrasonographic examination suggested a need for an earlier sample. A direct Coombs' test, red blood cell antibody elution, and identification were performed by the central hospital laboratory on the first fetal sample. Complete blood cell and reticulocyte counts were performed for each fetal sample. Not all values were available for all fetuses; therefore the totals are low in some of the data sets to be presented.

The technique used for cordocentesis has previously been described in detail.[16] In brief, a 22-gauge spinal needle was held in the ultrasonographic plane of a 5 MHz sector transducer with a fixed-needle guide (CIVCO, Kalona, Iowa). Pancuronium bromide (0.3 mg/kg estimated fetal weight intravenously or intramuscularly) was given when fetal movement was believed to pose a risk. We prefer the umbilical vein to the umbilical artery for sampling to minimize fetal morbidity. To minimize the risk of exacerbating mild disease, an effort was made to avoid puncturing the placenta when results of the maternal Coombs' test were relatively low (<128). In these instances maternal serum testing was repeated at 4-week intervals to identify a significant increase in the titer (two-tube or more dilution).

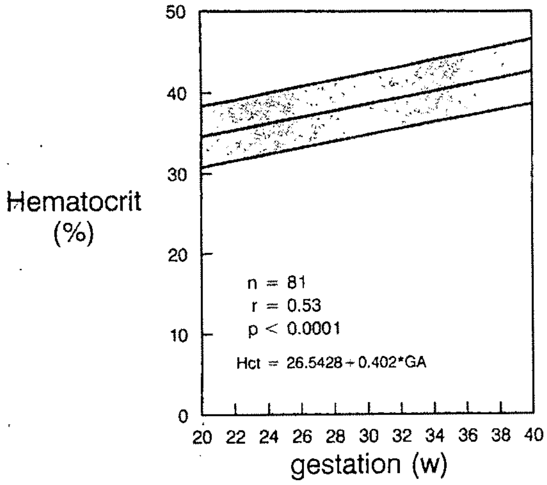

Fig. 1. Ninety-five percent confidence intervals for normal hematocrit values across gestational age *(GA)* (n = 81). *W*, weeks.

The control values were derived from fetuses referred for a variety of indications[16] who ultimately were shown to be free of disease. The 95% confidence intervals of the data were calculated for both the hematocrit count and the percentage of reticulocytes. Transfusion therapy was reserved for fetuses with severe anemia (defined as a hematocrit value <30%). A hematocrit value of 30% was <2.5th percentile for all control fetuses ≥20 weeks. The confidence interval for the hematocrit value was based on the linear regression of hematocrit value versus gestational age (r = 0.55, p < 0.0001). Because the correlation coefficient is fairly low, this type of calculation gave the widest normal range (Fig. 1). By taking this approach we believed we could avoid treating fetuses with borderline disease. For similar reasons we used the actual data rather than the regression (r = −0.54, p < 0.0001) to calculate the confidence intervals for the reticulocyte count across gestation. This conservative approach yields a narrower normal range (Table I). Thus we could be confident that any fetus said to have a normal count was indeed normal.

Table I. Normal fetal reticulocyte counts by gestational age

Wk	*n*	*2.5th percentile*	*Mean*	*97.5th percentile*
19.1-22	13	11.4	13.3	15.2
22.1-25	21	9.2	10.1	11.1
25.1-28	12	6.3	7.2	8.2
28.1-31	11	5.4	6.8	8.2
31.1-34	10	3.5	4.5	5.5
>34	3	3.4	4.3	5.3

Reticulocyte counts are percentage of red blood cells.

Table II. Serum antibodies numbering eight or more found in alloimmunized women

		*Hematocrit value <30%**	
Antibodies	*n*	*No.*	*%*
D	44	20	45
D,C	18	8	44
D,C,FyA	1	1	100
D,C,E	3	3	100
D,C,E,Kell	1	1	100
D,E	1	1	100
D,JkA	1	1	100
D,Kell	1	1	100
D,FyA	1	1	100
C	2	2	100
E	9	1	11
E,FyA	2	0	0
c	9	1	11
c,E	3	2	67
Kell	16	5	31
Kell,FyA,JkA	1	1	100
FyA	12	2	17
JkA	2	1	50
Hutch	1	1	100
TOTAL	128*	53	

*In one patient neither direct Coombs' test nor reticulocyte count was done.

All records were maintained prospectively in a computer data base. The results are presented as the mean ± 1 SD unless stated otherwise. Analyses included paired and unpaired Student's t tests, cross-tabulation, and stepwise multiple regression. A $p \leq 0.05$ was considered to indicate a significant difference.

Results

Through November 1990, 128 consecutive pregnant women who fulfilled the criteria were evaluated for alloimmunization to a variety of antigens associated with fetal hemolytic disease (Table II). Fifty-five percent were alloimmunized to the D antigen, although two thirds of these women had one or more additional immunoglobulin G antibodies present. The mean gravidity for the entire group was 3.5 ± 1.4 (range 1 through 9) and the parity was 1.9 ± 1 (range 0 through 5). The two primigravida women had become sensitized after transfusion; each had an anti-Kell titer of 256. Seventy percent of the patients had been pregnant 2 or more times.

Two hundred seventy-two diagnostic cordocenteses were performed. The mean gestational age at the time the first specimen was taken was 26 ± 4 weeks (range 18 to 34 weeks). Twenty-nine (23%) fetuses were antigen negative.

Fetal bradycardia occurred after 4% of the procedures, more commonly when the umbilical artery was punctured: 15% versus 2.8% ($p < 0.05$). One antigen-positive fetus was delivered by emergency cesarean section at 28 weeks because of prolonged bradycardia associated with cordocentesis. The infant was discharged from the nursery after having severe respiratory distress syndrome.

One affected fetus whose sample was taken at 21 weeks died 2½ weeks after the procedure and 3 days after ultrasonography had documented both viability and normal interval growth. Although the cause of death could not be determined at autopsy, there was no evidence that the death was procedure related.

We investigated whether the antenatal development of a hematocrit value <30% could be predicted from the fetal blood tests, beginning with the 53 fetuses who were initially seen with severe anemia ($n = 18$) or in whom severe anemia developed ($n = 35$). There was a positive relationship between the trimester in which the anemia developed and the result of the direct Coombs' test ($p < 0.05$, Table IIIA). Sixty-two percent of fetuses in whom severe anemia developed in the second trimester had direct Coombs' test results of 3+ or 4+. A similar trend was observed when the initial reticulocyte count was above the 97.5th percentile ($p = 0.07$, Table IIIB). The direct Coombs' titer was ≤2+ in each of the six instances in which the reticulocyte count was below the 2.5th percentile. Finally, there was a positive correlation between the direct Coombs' test result and the fetal reticulocyte count ($p = 0.015$). Thus the fetus at greatest risk for severe disease early in pregnancy either had a strong stimulus for hemolysis (i.e., a large amount of antibody coating the red blood cells) or had evidence of heightened red blood cell production.

When these same tests were applied to all antigen-positive fetuses, the relationships between the direct Coombs' test, reticulocyte count, and development of severe antenatal anemia were confirmed (Table IV). A reticulocyte count >97.5th percentile predicted the development of a hematocrit value <30% before delivery 83% of the time. Similarly, a direct Coombs' test result ≥3+ had a positive predictive value of 79% for the development of severe anemia.

On the basis of these relationships, we developed a scheme to manage the repetition of cordocentesis in the antigen-positive fetus. Four patterns were identified on the basis of the first blood sample taken. Management is summarized in Table V. The distribution of fetuses is summarized in Fig. 2. The 18 fetuses with a hematocrit value <30% when first seen were assigned to a pattern on the basis of their direct Coombs' test results and reticulocyte counts.

Pattern 1. Fetuses at lowest risk of developing a hematocrit value <30% before delivery (n = 11, 11%) had a normal hematocrit value, normal reticulocyte count, and a direct Coombs' test result that was either negative or trace positive when first sampled. These fetuses were monitored by serial ultrasonography. If the initial maternal antibody titer was low (<128), the indirect Coombs' test was repeated every 4 weeks in search of a twofold or more rise in titer. This occurred once. When the cordocentesis was repeated, the fetal hematologic profile was normal. These neonates were delivered at term. No neonate in this low-risk group was reported to have anemia at birth.

Pattern 2. Fetuses at intermediate risk of a hematocrit value <30% developing (n = 29, 31%) had a normal hematocrit percentage, a direct Coombs' titer of 1 or 2+, and a normal or low reticulocyte count when first sampled. Cordocentesis was repeated in 5 to 6 weeks. Ultrasonography was performed between procedures at 2- to 3-week intervals. This interval was initially selected so that transfusion therapy could be initiated if necessary before late in gestation (<32 weeks). As our experience has grown, we have transfused a fetus for the first time as late as 36 weeks rather than deliver prematurely. Blood sampling was not repeated a third time if the second hematocrit value and reticulocyte count were still normal. Instead, the pregnancy was monitored by ultrasonography every 2 weeks; delivery was planned for 38 weeks. Six (21%) fetuses in this group required transfusion late in gestation (29 to 36 weeks). In six of the seven, the anemia evolved slowly over 5 to 8 weeks. Two pregnancies requiring third-trimester transfusion were associated with maternal indirect Coombs' titers of 16 and 8.

Table IIIA. Relationship between results of initial direct Coombs' test and trimester in which severe anemia developed

Trimester hematocrit value <30th percentile	*Direct Coombs' test results*						
	–, Trace		*1+, 2+*		*3+, 4+*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*Total*
2	2	9	6	29	13	62	21
3	3	13	12	52	8	35	23
TOTAL	5		18		21		44

Not all data available for all fetuses.

Fisher exact test, p = 0.04 for risk of transfusion in the second trimester if direct Coombs' test results ≥3+ compared with all others.

Table IIIB. Relationship between initial reticulocyte count and trimester in which severe anemia developed

Trimester hematocrit value <30th percentile	*Reticulocyte count*						
	<2.5th percentile		*Normal*		*>97.5th percentile*		
	No.	*%*	*No.*	*%*	*No.*	*%*	*Total*
2	1	5	2	11	16	84	19
3	5	22	4	17	14	61	23
TOTAL	6		6		30		42

Not all data available for all fetuses.

Patterns 3 and 4. The third and fourth groups were at high risk of developing a hematocrit value <30%. They were characterized by either a normal hematocrit value with a direct Coombs' titer ≥3+ or a reticulocyte count >97.5th percentile or both (pattern 3, n = 49, 50%) or a mild anemia (i.e., hematocrit value ≥30% but <2.5th percentile for gestation) (pattern 4, n = 9, 9%). Each of the 18 fetuses with a hematocrit value <30% when first studied had, in addition to anemia, blood studies consistent with pattern 3. Cordocentesis was repeated at 1- to 2-week intervals. Ultrasonography was performed between procedures. Severe anemia developed in some of the fetuses; in 50% it did so in the second trimester. Eighty percent (n = 39) of fetuses with pattern 3 and 89% (n = 8) with pattern 4 had severe antenatal anemia.

Patterns 3 and 4 are similar but not identical. Several fetuses with pattern 4 had normal or low reticulocyte counts and a 1 to 2+ direct Coombs' titer. They would have been placed in a lower risk pattern and monitored less often if not for their low hematocrit values. Yet all

Table IV. Probability of anemia developing in antigen-positive fetus based on either initial reticulocyte count or direct Coombs' test

	Hematocrit value <30%		*Hematocrit value ≥30%*	
	No.	*%*	*No.*	*%*
Reticulocyte count > 97.5th percentile*	33	83	7	17
Reticulocyte count normal	7	23	24	77
Reticulocyte count < 2.5th percentile	6	43	8	57
Direct Coombs' test result −, trace†	5	31	11	69
Direct Coombs' test result 1+, 2+	23	49	24	51
Direct Coombs' test result 3+, 4+‡	22	79	6	21

Not all values available for all fetuses.

*Positive predictive value for reticulocyte count >97.5th percentile for severe anemia = 33 of 40 (83%); $\chi^2 = 26.1$ for cross-tabulation of reticulocyte groups, $p < 0.0001$.

†$\chi^2 = 10.6$ for cross-tabulation of direct Coombs' test groups, $p < 0.0001$.

‡Positive predictive value of direct Coombs' test resuts of 3+, 4+ for severe anemia = 22 of 28 (79%).

but one pattern 4 fetus became candidates for transfusion therapy before delivery. It may be that fetuses with pattern 4 were actually in a transition stage and if sampled earlier would have had a normal hematocrit value and been placed in either pattern 2 or 3. Two fetuses (one pattern 3 and one pattern 4) developed hydrops within 2 weeks of their first cordocentesis. In both instances, the placenta was posterior and the maternal indirect Coombs' titer was >1024 before evaluation. This experience stresses the importance of the interval ultrasonography. Three fetuses with patterns 3 or 4 in whom transfusion became necessary had a maternal indirect Coombs' titer ≤64.

Fetuses with anti-D antibody were significantly more likely to develop a hematocrit value <30% than those with a non-anti-D antibody (39/71 vs 16/57, $\chi^2 = 8.7$, $p = 0.003$). However, the patterns were equally predictive of the need for transfusion in D and non-D disease, suggesting that even though the stimuli for hemolysis varied, the responses to hemolysis were similar. The definitions of the patterns and the management of fetuses within each group are shown in Table V.

Although the data presented in the tables were based on all pregnancies evaluated through November 1990, the diagnostic scheme has remained unchanged since August 1989. This allowed us to examine the performance of the protocol prospectively in the 44 patients evaluated from September 1989 to the close of the study November 1990. In this group, 8 (18%) fetuses were antigen negative. The pattern distribution of antigen-positive fetuses and the incidence of transfusion were pattern I: $n = 7$, 0 (0%) transfused; pattern II: $n = 15$, six (40%) transfused; pattern III: $n = 13$, 8 (62%) transfused; and pattern IV: $n = 1$, 1 (100%) transfused.

Comment

Obtaining a fetal blood sample permits an accurate measurement of the fetal hematocrit. We have demonstrated that by measuring the indicators that reflect the potential for hemolysis (direct Coombs' titer) and the fetal response to hemolysis (the hematocrit and the reticulocyte count) nonanemic fetuses at greatest risk of developing anemia can be prospectively identified. Our experience with the direct evaluation of fetal hemolytic disease supports the findings of a smaller series by MacKenzie et al.[17]

We have constructed four patterns of fetal hemolytic disease that permit stratification on the basis of a fetus's risk of developing a significant antenatal anemia. In all, 31% of pregnancies studied required only one cordocentesis (29 antigen-negative fetuses plus 11 pattern 1 fetuses), and another 18% required only two procedures (23 fetuses with pattern 2 not requiring transfusion therapy). Eighty-five percent of the remaining fetuses had or developed a hematocrit value <30% and were candidates for transfusion therapy.

The antigen-positive fetuses at lowest risk of developing anemia (pattern 1) had a normal hematocrit value and reticulocyte count plus a negative to trace-positive direct Coombs' test result. These pregnancies were followed with periodic ultrasonography for the detection of hydrops. Otherwise, the women were delivered at term. To date, no fetus in this group has been reported to have been born with anemia.

Included in the intermediate-risk group (pattern 2) were several fetuses who failed to respond to their low-grade hemolysis with a reticulocytosis. Surprisingly, the reticulocyte count was actually below the 2.5th percentile for gestation in some of these fetuses. It is unlikely that the low reticulocyte count resulted from hemolysis of the reticulocyte before it entered the circulation because the fetal hematocrit value declined slowly. When transfusion therapy for this group was indicated, it was most often in the third trimester. It would appear that for some yet-to-be-defined reason, these fetuses have a blunted erythropoietic response to anemia.

In fetuses at greatest risk of developing anemia, the hematocrit value was already <2.5th percentile but >30% when first evaluated (pattern 4), or the hematocrit value was in the normal range but was associated

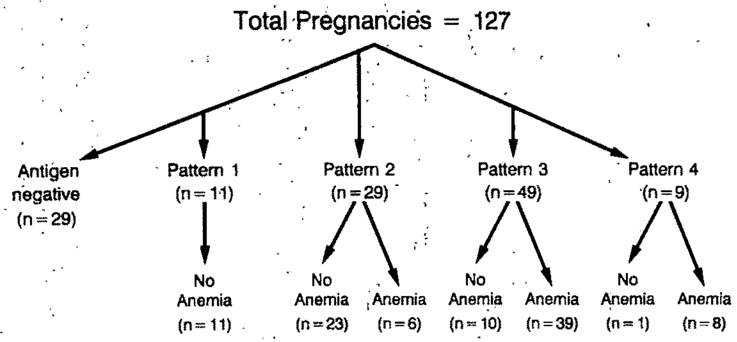

Fig. 2. Distribution of fetuses by hematologic pattern and development of severe anemia (hematocrit value <30%).

Table V. Criteria for repeat cordocentesis if affected fetus

Pattern	*Hematocrit*	*Reticulocytes*	*+ / − Direct Coombs' test result*	*Interval for cordocentesis (wk)*	*Interval for scan (wk)*	*Comments*
1	Normal	Normal	−/trace	—	4	Repeat if initial maternal indirect Coombs' test result < 128 and twofold increase documented
2	Normal	Normal or <2.5th percentile	1+/2+	5-6	2	Do not repeat after 32 wk if unchanged; delivery at term
3	Normal	>97.5th percentile	3+/4+	2	1	Continue through 34 wk if hematocrit value stable; deliver at 37-38 wk if not transfused
4	<2.5th percentile but >30%	Any	Any	1-2	1	Repeat as long as hematocrit criteria fulfilled; deliver with pulmonary maturity if not transfused

with either a reticulocyte count above the 97.5th percentile or a strongly positive direct Coombs' test result (pattern 3). Ninety-six percent of fetuses who developed or who were initially seen with anemia in the second trimester had either pattern 3 or 4. Their risk was reflected in the frequency of repeat sampling.

In spite of the high specificity of fetal blood testing, it is clear that the fetal response to alloimmunization was not completely defined by the direct Coombs' test result and the reticulocyte count. For example, three fetuses (one each with patterns 2 through 4) responded to the disease in the third trimester with an increased reticulocyte count. They ultimately were delivered at term with both a normal hematocrit value and reticulocyte count. These observations stress the importance of not initiating transfusion therapy just because the hematocrit value has fallen below the 2.5th percentile. Further, these tests do not predict postnatal hyperbilirubinemia. The fetus with pattern 4 who did not have severe anemia required two double-volume exchange transfusions for postnatal hyperbilirubinemia. It is possible that the addition of other parameters, such as fetal serum bilirubin concentration, might further refine the predictive value of the patterns and extend it into the postnatal period.

The positive predictive value of the reticulocyte count for severe disease observed here differs from that reported by Nicolaides et al,[18] who studied 29 pregnancies. These authors did not find the reticulocyte count to be of value in predicting the severity of fetal hemolytic disease. The difference in conclusions may reflect a different referral pattern, smaller sample size, reliance on smooth percentiles derived from regression with a parameter not tightly reflective of gestational age, or reticulocyte counts not corrected for gestational age.

Our data suggest that cordocentesis is an acceptable alternative to serial ΔOD_{450} measurements. Moreover, it offers several advantages. Amniotic fluid studies require a minimum of two specimens to establish a trend and lack specificity in identifying the fetus with severe anemia.[3,12,13] In contrast, only half of the pregnancies monitored by fetal blood sampling required more than two diagnostic procedures. Because the ambiguity of using the ΔOD_{450} as a predictor of fetal anemia was eliminated, women whose fetuses were not anemic could be delivered at term when the postnatal hyperbilirubinemia was more readily handled and better tolerated by the neonate and more easily treated.

Although the ΔOD_{450} reflects the concentration of a hemoglobin breakdown product, its utility is based on its relationship to the hematocrit value.[1,3,13] In the absence of anemia, severe fetal pathologic sequelae to alloimmunization are not seen. We did not randomly compare the two systems of diagnosis because our initial experience[13] with such a comparison and the larger experience of Nicolaides et al.[12] indicated that the relationship between hematocrit value and ΔOD_{450} was inexact. In agreement, Frigoletto et al.[19] observed that 37% of fetuses with ΔOD_{450} in zone III had hematocrit values >30% at birth.

One concern about cordocentesis is that it may enhance maternal sensitization more than amniocentesis. However, the anterior placenta can usually be avoided with cordocentesis as well as it can be with amniocentesis. Moreover, we have no evidence that cordocentesis performed with a needle guide to prevent lateral movement intensifies maternal sensitization to a greater degree than simple amniocentesis. The increase in maternal serum α-fetoprotein after cordocentesis in our unit is not different from the increase reported after amniocentesis.[20] Only one patient in this series who began with an indirect Coombs' test result <128 at the outset had a two-tube rise in titer. Although we did not routinely measure the maternal indirect Coombs' titer serially when it was >128 on referral, increases probably occurred as they do after amniocentesis.

Another concern is the greater risk associated with cordocentesis compared with ultrasonography-guided amniocentesis. In our experience with >800 cordocenteses, there has yet to be a procedure-related death of an appropriately grown fetus. Morbidity can occur. In this series, one fetus was delivered prematurely because of associated prolonged bradycardia. In our experience bradycardia is the major risk of cordocentesis.[16] If bradycardia could be prevented or effectively treated, we believe the risks from amniocentesis and cordocentesis would be indistinguishable if performed by an experienced operator. At this time it is unsettled as to whether the reduction in the number of invasive procedures clearly achieved by the enhanced specificity of fetal blood sampling compensates for any increased risk per procedure. It appears reasonable for the primary physician to begin the evaluation with amniocentesis (assuming a low likelihood of paternal heterozygosity and no history of a severely affected fetus) and then to refer the patient to an appropriate center for cordocentesis if the ΔOD_{450} is in zone II or higher.

Cordocentesis and intravascular transfusion are deceptively simple. In reality, they require an experienced team and a laboratory capable of performing a variety of tests on a small volume of specimen. Our series was drawn from a delivery base approaching 60,000 per year. It is not practical from the standpoint of personnel, costs, and experience for every hospital to offer a similar service. These facilities must be regionalized to maximize both safety and efficacy.

Evaluation of fetal hemolytic disease with a blood sample permits identification of the fetus at high risk of having anemia.

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Steroid therapy for hydrops associated with antibody-mediated congenital heart block

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Evaluation of fetal bradycardia noted during a routine prenatal visit at 24 weeks' gestation in a mother without symptoms showed an antibody-mediated heart block with anti-La (SSB) antibody present. The fetus had a pericardial effusion and ascites. After maternal steroid therapy, the ascites resolved and the pericardial effusion was substantially diminished. Steroid therapy may be a helpful adjunct for treatment of a premature infant with hydrops as a result of antibody-mediated heart block. (AM J OBSTET GYNECOL 1991;165:553-4.)

Key words: Heart block, SSA, SSB, antibodies, steroid therapy

Complete congenital heart block associated with maternal anti-SSA or SSB antibodies may occasionally cause hydrops fetalis. The hydrops has been thought to occur as a result of low fetal cardiac output. We report a fetus with antibody-mediated heart block in whom maternal steroid therapy was associated with reversal of ascites and improvement of the pericardial effusion.

Case report

A 36-year-old primigravid patient was first seen at 15 weeks' gestation, at which time ultrasonography showed normal fetal anatomy. Amniocentesis done for maternal age showed 46,XX. At 24 weeks' gestation, during a routine prenatal visit, the fetal heart rate on auscultation was 60 beats per minute. Ultrasonography showed biometry consistent with dates, bradycardia with a rate of 58 beats per minute, a moderate pericardial effusion, and minimal ascites. Detailed views of the fetal heart did not reveal structural anomalies.

The mother was without symptoms and without a history of connective tissue disease. She was found to have a positive antinuclear antibody at 1:640 and positive anti-La (SSB) antibody with a titer of 1:128. Anti-SSA antibody was not present. Steroid therapy was discussed, and she was treated with betamethasone 12.5 mg intramuscularly daily for 2 days, followed by 60 mg of prednisone daily. After 1 week, repeat ultrasonography showed no ascites and a decreased pericardial effusion, although the fetal heart rate was unchanged. The prednisone was empirically tapered every 2 weeks; at 32 weeks' gestation she was taking 10 mg daily. The fetal pericardial effusion became minimal but did not entirely resolve. No fetal growth was seen between 33 and 35 weeks' gestation by ultrasonography, and the

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patient was delivered of an infant by cesarean section at 35 weeks.

The infant weighed 2200 gm, had Apgar scores of 8 and 8 at 1 and 5 minutes, respectively, and did well without cardiac pacing. An electrocardiogram showed third-degree atrioventricular block, and the rate varied from 52 to 75 beats per minute. At 5 weeks of age mild congestive heart failure developed with a heart rate in the low 50s; a pacemaker was placed. The infant is doing well at 6 months of age.

Comment

Hydrops that develops in a fetus with antibody-mediated heart block has traditionally been thought to be caused by low-output cardiac failure. Recently, however, an immune mechanism has been proposed for these serous effusions, and two fetuses have been treated in utero with steroid therapy, with improvement of the fetal condition.[1, 2]

Immune-mediated hydrops may be caused by inflammation of the fetal peritoneum and pericardium. Steroid therapy in such cases may decrease this inflammation. Because prednisone does not cross the placenta in substantial amounts, it may work by decreasing maternal antibody levels.

Although one must be cautious in attributing the resolution of hydrops to steroids, the temporal association between therapy and fetal improvement in this and two other reported cases[1, 2] is suggestive. The dosage and duration of therapy in this case was empiric, and it is unclear whether the use of two different steroids (betamethasone and prednisone) is indicated. We based our therapy on the report of Richards et al.[2] who used both steroids in a similar clinical situation.

Maternal steroid therapy may be a useful in utero therapy for the premature fetus with antibody-mediated congenital heart block in which hydrops develops.

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Prognostic factors and complication rates for cervical cerclage: A review of 482 cases

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Charts from 482 singleton pregnancies undergoing cerclage placement in patients over a 6-year period were reviewed. Cervical dilatation at time of surgery, number of previous spontaneous losses, and gestational age at placement were key determinants of outcome. The most frequent complication, premature rupture of the membranes, ultimately occurred in 38% of patients. The 6.6% infection rate was increased in patients undergoing emergent versus elective surgery (12.7% vs 4.7%, $p < 0.005$) and in patients at increased dilatation at time of surgery (5.7% for ≤2 cm vs 41.7% for >2 cm, $p < 0.005$). McDonald and Shirodkar procedures had similar obstetric outcomes in patients undergoing their first cerclage. The subgroup of patients with prior cerclage surgery showed improved birth weight when the Shirodkar procedure was performed instead of the McDonald cerclage (mean birth weight 3020 vs 2470 gm, $p < 0.005$). An increased rate of primary cesarean section was found in the Shirodkar group (31% vs 17%, $p < 0.005$). Complication rates and pregnancy outcome appear to reflect cervical dilatation more than gestational age at time of surgery. (AM J OBSTET GYNECOL 1991;165:555-8.)

Key words: Cerclage, incompetent cervix

Cervical cerclage has become an established treatment for cervical incompetence. It is often unclear in individual cases whether the benefits of the procedure outweigh its potential complications. There is also continued disagreement regarding choice of surgical technique.

The role of cerclage placement with advanced gestation or dilatation remains controversial. Schwartz et al.[1] described 74 cases of cervical cerclage with relatively low rates of cervical laceration cesarean section and no significant difference in infection rates between early and late cerclage placement (after 18 weeks' gestation). Charles and Edwards[2] reported a 2.6-fold increase in incidence of chorioamnionitis and a threefold increase in premature rupture of membranes (PROM) before 32 weeks' gestation when the procedure was performed after 18 weeks. They concluded that the optimum time of placement was between 14 and 18 weeks' gestation. Other series have noted an increased frequency of abdominal delivery,[3] high overall rates of PROM,[4, 5] and high rates of chorioamnionitis[5, 6] complicating all pregnancies managed with cerclage. A marked increase in cervical laceration rate (23.8%) has been noted among patients undergoing emergency procedures.[3] Although it has been reported in small series that advanced cervical dilatation (>3 cm) is associated with a worse prognosis,[7, 8] this factor is not considered in most studies. Some authors have advocated cerclage placement for patients with bulging membranes before 25 weeks gestation.[9, 10]

Surgical technique, as well as patient selection, remains controversial. In Harger's[11] review of 251 cases, McDonald and Shirodkar procedures had equivalent success rates and morbidity. This finding was confirmed by Cardwell[12] in a study of 129 pregnancies. In spite of this, many obstetricians routinely advocate one technique in preference to the other.

Previous studies have been limited by the number of cases, collection over excessively long periods of time, or not considering one or more important prognostic factors. Prospective, randomized studies examining cerclage have excluded patients with cervical dilatation, shortening, or previous clerclage.[4] There is wide acceptance of cervical cerclage placement for treatment of the incompetent cervix. Withholding cerclage therapy with documented cervical incompetence may be considered unethical, making completely randomized studies impossible. Consequently, the relationships between gestational age, dilatation, and type of procedure to eventual results remain unclear. The purpose of this study is to perform a comprehensive evaluation of the determinants of outcome for cerclage placement. It is hoped that this information will assist in selection of patients and surgical technique.

Material and methods

We reviewed medical records of 437 patients having one or more cerclage procedures during singleton

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Table I. Characteristics of 482 pregnancies managed with cerclage

Variable	*Median*	*Range*
Maternal age (yr)	28	16-42
Gravidity	4	1-14
Parity	1	0-9
Number of previous spontaneous abortions	2	0-10
History of prior cerclage	39.0%	—

Table II. Relationship between dilatation at time of cerclage placement and pregnancy outcome (mean ± 1 SD)

Dilatation (cm)	*No.*	*Interval to delivery (wk)*	*Gestational age at delivery (wk)*
0	410	19.2 ± 6.6	35.6 ± 6.3
1	41	12.6 ± 7.5	33.3 ± 7.1
2	19	8.8 ± 8.2	28.7 ± 9.4
≥3	12	1.2 ± 2.1	23.5 ± 4.1

Table III. Relationship between gestational age at time of cerclage placement and pregnancy outcome in patients with no dilatation at time of cerclage (mean ± 1 SD)

Gestational age at cerclage (wk)	*No.*	*Interval to delivery (wk)*	*Gestational age at delivery (wk)*
12-13	64	22.1 ± 7.4	34.6 ± 7.4
14-15	107	20.5 ± 6.8	34.8 ± 6.8
16-17	120	19.3 ± 6.4	35.7 ± 6.3
18-19	50	17.7 ± 5.8	36.1 ± 5.8
20-23	52	16.2 ± 4.0	37.2 ± 4.0
24-26	17	11.8 ± 4.4	36.8 ± 4.3

pregnancies between January 1982 through December 1987 at our hospitals. Surgeries performed at gestational ages <12 weeks or <26 weeks were not included in the analysis because they were believed to reflect unacceptable variation in the quality of care. On the remaining 482 procedures maternal age, gravidity, parity, previous spontaneous losses at <24 weeks' gestation and history of previous cerclage placement were abstracted. The gestational age of cerclage placement and type of cerclage procedure (Shirodkar vs McDonald) were determined. Indications were classified as elective when based on past obstetric history or emergent when cervical changed prompted operation. Cervical dilatation at the time of the procedure was recorded in the latter group. Outcome variables included gestational age at delivery, birth weight, PROM, and clinical chorioamnionitis (temperature >38° C and uterine tenderness). Other data included the mode of delivery and complications such as cervical lacerations. Unfortunately, information regarding neonatal morbidity and mortality is not available on these specific pregnancies. About 60% of patients at our hospitals have private attending physicians and the remainder are assigned to staff in the department. Resident physicians under attending supervision perform most cerclage procedures on both private and staff services. A variety of suture material were used secondary to the large number of practitioners. Almost always a single stitch was placed; tocolytic and antibiotic prophylaxis were not routinely used.

Gestational age at delivery and the interval from cerclage placement to delivery were analyzed with stepwise multiple regression. The Student *t* test was used to evaluate continuous variables such as birth weight differences between two groups. Discriminant function analysis was used to evaluate factors associated with PROM, a dicotomous outcome. Other nominal variables such as chorioamnionitis were analyzed with χ^2. To adjust for the multiple comparisons made in this study, $p < 0.005$ was considered significant.

Results

Characteristics of the pregnancies are presented in Table I. The relatively older maternal age appears to reflect the incidence of reproductive problems documented by a median number of spontaneous abortions exceeding the median parity. It should be noted, however, that the number of previous spontaneous abortions indicates that many patients had none or only one previous loss at the time of cerclage placement. The mean gestational age at placement was 17 weeks. Of the 482 procedures, 364 were elective; the remainder had documented cervical change. Cervical dilatation at time of placement in the latter group ranged from 0 to 4 cm.

The mean interval to delivery was 17.7 weeks with a range of 0 to 30 weeks; the mean gestational age at delivery was 34.8 weeks with a range of 12 to 43 weeks. Tables II and III are descriptive tables that show an interesting contrast between dilatation and gestational age at cerclage placement as they relate to gestational age at birth and interval to delivery. Advanced dilatation was associated with increasing prematurity and decreased interval to delivery. Among those with no dilatation at time of cerclage, earlier gestational age at placement resulted in longer intervals to delivery, as would be anticipated. In these procedures an increasing mean gestational age at delivery was noted with advancing gestational age at time of cerclage. It should be noted placement late in gestation excluded cases that aborted early in pregnancy. The results of the stepwise multiple regression analysis of this data are summarized in Table IV. Cervical dilatation at time of cerclage, gestational age at placement, and number of previous pregnancy losses at <24 weeks' gestation were key de-

Table IV. Results of stepwise multiple regressions relating prognostic factors of obstetric outcomes

Variable	Gestational age at delivery			Interval to delivery		
	F	β	p Value	F	β*	p Value
Dilatation (>2 cm)	59.3	−0.40	<0.001	145.2	−0.34	<0.001
Gestational age at cerclage	9.3	0.17	<0.001	54.8	−0.39	<0.001
Prior spontaneous abortions (<24 wk)	20.3	−0.17	<0.001	16.1	−0.15	<0.001
History of prior cerclage	0.13		NS	0.13		NS
Type of cerclage	0.13		NS	0.13		NS
Indication (elective vs emergent)	0.0		NS	0.0		NS

β, Standardized regression weight, which indicates direction and relative magnitude of effects (not calculated for insignificant variables).

Table V. Factors affecting the rate of premature rupture of membranes in cerclage patients

Variable	Premature rupture of membranes		
	Rate	F	p Value
Dilatation (>2 cm)	75%	6.1	NS (<0.05)
Gestational age at cerclage	—	1.31	NS
Prior spontaneous abortions (<24 wk)	—	0.55	NS
History of prior cerclage placement	35.7%	0.19	NS
Shirodkar cerclage	33.3%	0.01	NS
Indication (elective)	36.3%	0.59	NS

terminants of pregnancy outcome. Prior cerclage placement, type of cerclage, indication, gravidity, and parity did not appear to be significant after accounting for these factors.

The most frequent complication noted was PROM, ultimately occurring in 38.2% of pregnancies. The analysis summarized in Table V suggests that dilatation at cerclage placement may be the most important factor relating to incidence of PROM. When dilatation was removed from the analysis no other factors appeared to have a significant impact on PROM rates. In 116 of the 184 patients with PROM delivery occurred at <37 weeks' gestation; 48 were delivered of infants at <27 weeks. The infection rate (6.6% overall) differed according to cervical dilatation [28/451 (6.2%) for ≤2 cm vs 5/12 (41.7%) for >2 cm, $p < 0.005$] and indication for procedure [emergent 17/118 (14.4%) vs elective 19/364 (5.2%), $p < 0.005$].

The incidence of cervical lacerations for emergent procedures was not significantly different from that of elective procedures. Although there was a trend for cervical lacerations to be more common in patients who underwent cerclage placement after 18 weeks' gestation, it was not statistically significant [15/140 (10.7%) vs 16/342 (4.7%) $p < 0.02$]. Cervical fistulae in three patients developed after cerclage placement.

McDonald procedures were performed in 377 pregnancies; Shirodkar technique was used for the other 105 cases. All patients dilated >1 cm at the time of operation underwent McDonald cerclages. All the McDonald sutures were removed before or at the time of presentation to labor and delivery. Four patients with a Shirodkar cerclage underwent primary cesarean sections with no attempt at cerclage removal (one patient required removal after delivery because of infection at the cerclage site). Attempts were made to remove the remainder of the Shirodkar sutures before delivery. The overall cesarean rate in this series was 31.8%. The primary cesarean rate was 31% with Shirodkar cerclage compared with 17% for McDonald technique ($p < 0.005$). This was attributable to cesarean deliveries performed for cervical dystocia in 12/105 cases with Shirodkar cerclages compared with 10/377 in the McDonald group ($p < 0.005$). Rates of primary cesarean sections performed for other indications and repeat cesarean sections were similar for the two cerclage procedures. There were 182 patients undergoing repeat cerclage procedures. Within this subgroup, pregnancies managed with Shirodkar cerclage instead of McDonald procedure appeared to have improved mean birth weight (grams) (3020 ± 951 vs 2470 ± 1100, $p < 0.005$) and gestational age at delivery (weeks) (37.0 ± 5.6 vs 34.5 ± 6.5, $p < 0.03$).

Comment

Interpretation of these findings have led us to several conclusions. The most important determinants of gestational age and interval to delivery appeared to be cervical dilatation at the time of procedure, gestational age at placement, and the number of previous losses. Cervical dilatation >2 cm at cerclage was associated with more severe prematurity and a greater risk of PROM, but no increase in the frequency of cervical lacerations. Shirodkar technique resulted in an increased primary cesarean section rate. Although there was no demonstrable advantage over the McDonald procedure in patients undergoing their initial cerclage surgery, patients with previous cerclage appeared to have improved birth weights when Shirodkar technique was used.

Improved pregnancy outcomes with cerclage placement before 18 to 20 weeks' gestation,[2, 5, 11, 12] and a twofold to three-fold increase in PROM and chorioamnionitis with placement after 18 weeks have been reported.[2] These considerations have been part of the rationale for advocating surgery between 14 and 18 weeks' gestation. Our increased rate of PROM (38%) appeared related to the large number of emergency placements in the face of cervical dilatation; advancing gestational age did not have as strong an influence.

Our 6.7% incidence of cervical lacerations is consistent with previous literature.[1, 3, 11, 13, 14] Contrary to previous reports, emergent procedures were not at increased risk and dilatation or history of prior cerclage did not appear to be factors.

Increased cesarean rates have been noted in most cerclage series,[3, 4, 13-15] particularly among patients with Shirodkar procedures.[11] Our current results confirm in a larger series that this pattern has continued. Much of the use of abdominal delivery can be directly attributed to the cerclage procedure. In addition to the practice of performing primary elective cesarean on patients with a Shirodkar cerclage in place, there is an increased incidence of cervical dystocia.[1, 6, 11, 16] The rate of cervical dystocia was 2.6% in patients with a McDonald cerclage and 11.4% with the Shirodkar procedure. This rate of cervical dystocia (4.3% overall) rose to 14.0% (12/86) in patients with a Shirodkar cerclage who were given a trial of labor. On the basis of this finding, we believe that use of modified Shirodkar technique with removal during labor may cause more scarring of the cervix, leading to a greater risk of cervical dystocia and cesarean section. With the exception of cesarean rates, numerous series have reported similar obstetric outcome with McDonald and Shirodkar procedures.[3, 7, 9, 12] We found this to be true only in patients with no history of prior cerclage.

We would like to emphasize the limitations inherent to this and all other retrospective studies. A prospective randomized trial is needed to make definitive conclusions regarding the advisability of cerclage in cases in which the benefits and risks appear to be closely balanced. A similar study is needed to determine the optimal surgical technique. Nonetheless, we believe these findings have several implications for clinical practice and for future research.

When considering cerclage, gestational age appears to be a less important factor than cervical dilatation. Certainly, patients and their physicians should be aware of the poor prognosis after emergency placement in a dilated cervix; the risks may outweigh the benefits in many cases. The belief that modified Shirodkar technique with removal of the cerclage is comparable to the McDonald procedure appears to be mistaken; the need for abdominal delivery is still increased because of a greater likelihood of cervical dystocia. The role of placement of a permanent Shirodkar cerclage at time of initial cerclage surgery was not examined. Certainly, if the plan is for modified Shirodkar cerclage with removal at delivery we advocate the McDonald procedure as the initial cerclage procedure when possible to avoid the morbidity and mortality associated with cesarean delivery. In patients undergoing repeat cerclage the Shirodkar appears to offer improved birth weights and higher gestational age at delivery. It may be warranted to use this technique more frequently in this subgroup of patients.

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Incidental appendectomy during videolaseroscopy

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One hundred incidental appendectomies were performed in patients undergoing operative laparoscopy (videolaseroscopy) for evaluating and treating various major diseases of the reproductive organs. Except for a fever resolving within 24 hours in one case and mild periumbilical ecchymosis, there were no intraoperative or postoperative complications. All patients were discharged within 24 hours of surgery. Average hospital stay was 14 hours. All cases have been followed up for a minimum of 8 months. We believe any risk associated with elective appendectomy as reported here is minimal and outweighed by the benefits of eliminating future emergency appendectomy, simplifying future differential diagnosis of pelvic pain and removing unsuspected abnormality found in the appendix. (AM J OBSTET GYNECOL 1991;165: 559-64.)

Key words: Incidental appendectomy, videolaseroscopic appendectomy, pathology in elective appendectomy, endometriosis, laparoscopy, operative laparoscopy

Although elective, prophylactic, or incidental appendectomy during surgery for treating other abdominal pathologic conditions is not a settled issue,[1] many physicians, particularly in obstetrics and gynecology, advocate the practice with some qualifications and unless there are specific contraindications.[2-4] The emergence of diagnostic and operative laparoscopy as a viable option to laparotomy over the past two decades[5-14] has followed development of safe procedures for using instruments and tools, wedding endoscopy with electrocautery and using laser and video technology.[8, 12-14] Appropriate applications have involved a variety of gynecologic disorders and procedures, including incidental appendectomy. We review here the results of 100 consecutive appendectomies performed during videolaseroscopy in which diseases of the reproductive organs were being evaluated and treated.

Material and methods

One hundred patients undergoing videolaseroscopy with the carbon dioxide laser for evaluation and treatment of endometriosis with diverse pelvic pathologic conditions were studied. Among them were 14 who requested appendectomy before they gave informed consent. Patients' ages ranged from 17 to 45 years. Fifteen were <25 years old; 53 were between 25 and 35; and 32 were >35 years old. General endotracheal anesthesia was used in all cases, and each patient received a 2.0 gm intravenous prophylactic dose of cefoxitin sodium (Mefoxin; Merck Sharp and Dohme, West Point, Pa.) or 100 mg of Vibramycin (Pfizer, New York).

Current procedures[8-14] evolved from efforts initiated in 1983 using video-augmented instrumentation at hand to develop optimal techniques for laparoscopic surgery, including appendectomy (Fig. 1). Those employed in the present study involved routine induction of pneumoperitoneum and insertion of the laparoscope intraumbilically. Subsequently three 5.0 mm suprapubic punctures were made: one in the lower right quadrant, one in the lower left quadrant, and one in the midline, two fingerbreadths above the pubic symphysis. These trocar punctures allowed the introduction of ancillary instruments such as grasping forceps, endoloop suture applicators, and units for suction and irrigation and bipolar electrocautery. Hemostasis was accomplished with the laser beam or by electrocautery.[8-10]

Gynecologic procedures taking from 30 minutes to 3 hours were performed. Initial evaluation revealed 58 cases of pelvic endometriosis; 10 others had pelvic adhesions, and 32 had endometriosis and adhesions of the pelvis and abdomen, including severe involvement of the descending colon and rectovaginal septum. Major surgery included carbon dioxide laser ablation or excision of peritoneal and ovarian endometriosis, ovarian cystectomy, salpingoovariolysis, salpingectomy,[10a] salpingo-oophorectomy, salpingoneostomy, adnexectomy, myomectomy, enterolysis, ureterolysis, ureteroneocystostomy, and partial colon resection for the treatment of endometriosis.[10b]

On completion of thorough treatment of the pelvic pathologic condition, preparation for appendectomy included inserting grasping forceps through the lower right-quadrant trocar sleeve and passing the bipolar electrocoagulator and the suction irrigator through the midline and lower left-quadrant punctures, respec-

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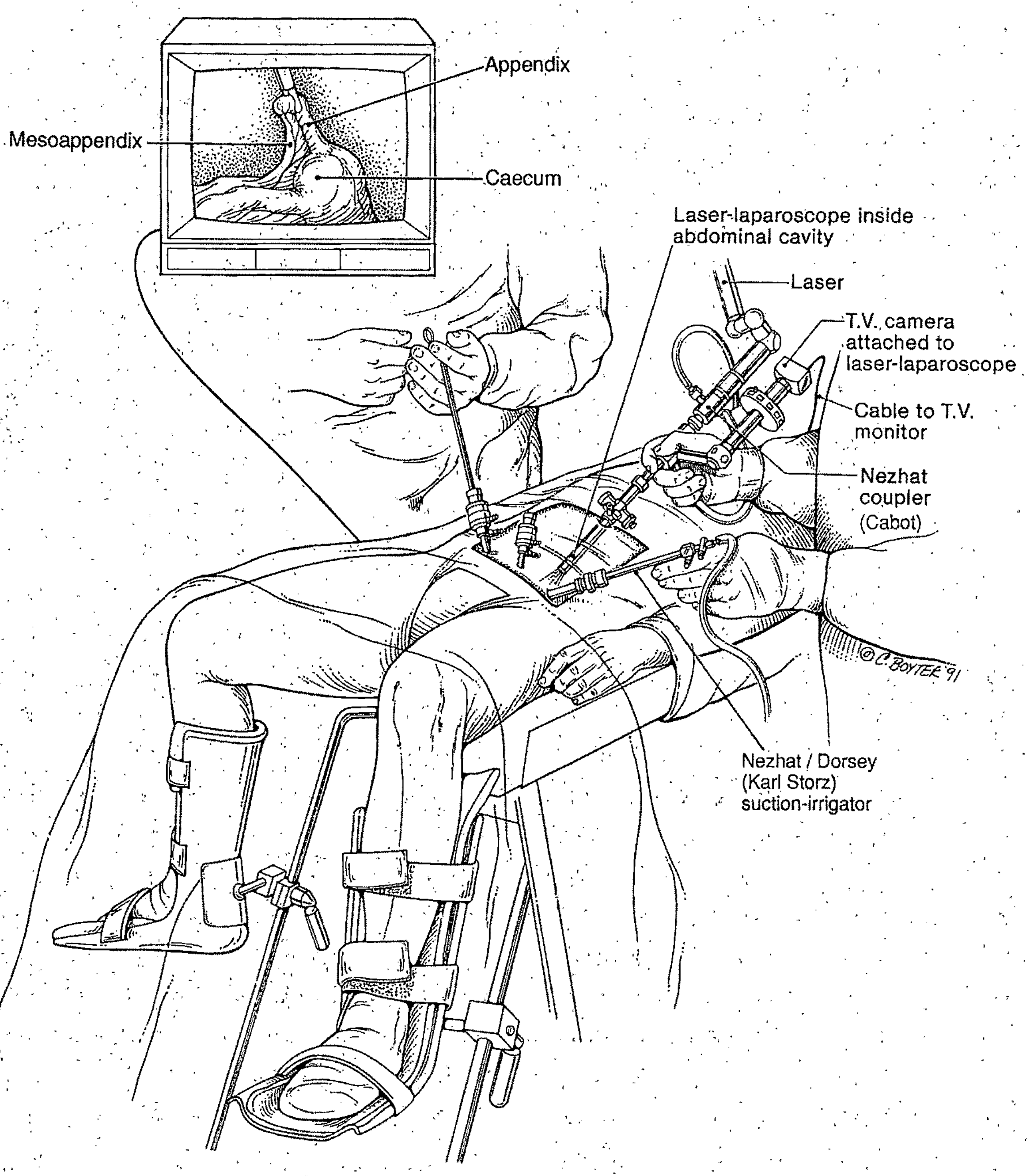
Appendix
Mesoappendix
Caecum
Laser-laparoscope inside abdominal cavity
Laser
T.V. camera attached to laser-laparoscope
Cable to T.V. monitor
Nezhat coupler (Cabot)
©C.Boyter '91
Nezhat / Dorsey (Karl Storz) suction-irrigator

Fig. 1. Operating room setup for videolaseroscopy.

tively. Then, with the video laparoscope system in the umbilical channel, the appendix was located. Subsequently, a direct lens-laser coupler was attached,[9-12] and the carbon dioxide laser beam through the operating channel of the laparoscope (Fig. 1) was used as a long scalpel and to coagulate small blood vessels. The appendix was mobilized and examined after lysis of periappendiceal or pericecal adhesions as necessary. This was done carefully in case there was attachment to the lateral pelvic wall or retrocecal appendix. Next, the bipolar cautery and the carbon dioxide laser were used sequentially to coagulate and cut the mesoappendix, ½ centimeter away from the ileocecal area.

At this point the bipolar coagulator was withdrawn and the endoloop applicator was inserted through the suprapubic midline puncture. Two chromic endoloop sutures (Ethicon, Somerville, N.J.) were passed to the base of the appendix, 3 to 5 mm from the cecum, and then tied one on top of the other. Both suture ends were cut with the carbon dioxide laser. A third endoloop suture was applied, 1 cm distal to the other sutures, and then cut long, leaving a 15 cm tail to facilitate retrieval should the appendix inadvertently fall into the pelvic well. With the carbon dioxide laser the appendix was cut between the sutures mentioned (Figs. 2 to 5). Luminal portions of the appendiceal stump and the

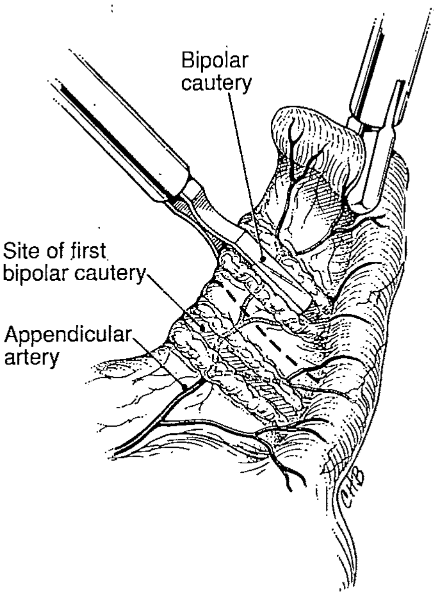

Fig. 2. Mesoappendix being coagulated.

removed appendix were seared with the carbon dioxide laser and rinsed with one or two drops of povidone-iodine (Betadine, Purdue Frederick, Norwalk, Conn.) (Fig. 6). The tissues were then copiously irrigated with lactated Ringer's solution containing heparin, 5000 U/L.

The appendix, which was subsequently sent to the laboratory, was removed from the abdomen either by use of a long, grasping forceps passed through the operating channel of the laparoscope or suprapubically with the short grasper; if appropriate, an appendix extractor was used through the sleeve of a 10 mm trocar, replacing the central 5 mm one. Instruments, possibly contaminated, were removed from the surgical area. No adjunctive therapy was added to the pelvic cavity. Last, the appendiceal and other operative sites were inspected for hemostasis by completely emptying and reinsufflating the carbon dioxide gas into the abdominal cavity and finally irrigating with lactated Ringer's solution.

Appendectomies were accomplished in from 4 to 21 minutes. Postoperative instructions included avoiding solid food for 24 hours; otherwise, instructions were those routinely used for gynecologic procedures, including an outpatient evaluation the day after surgery.

Results

No major intraoperative complications occurred in any patient. All patients were discharged from the hospital within 24 hours of surgery. Seven remained overnight because of patient preference or surgery late in the day. The average hospital stay was 14 hours.

In one patient a temperature of 101° F developed the evening after surgery and she was observed overnight. No acute abdominal signs occurred, and the abdomen was soft the next day. White blood cell count was 7500/mm^3, with no shift in the differential. The temperature fell, and the patient was discharged from the hospital free of fever.

All patients resumed a regular diet the day after surgery. Except for mild periumbilical ecchymosis, no apparent postoperative complications have occurred in the study group. All have thus far been followed up for a minimum of 8 months.

Fifty-two of the 100 removed appendixes were reported as normal. These include 12 instances of focal fibrous obliteration of the lumen of the appendix and 14 patients requesting appendectomy, as noted earlier. Pathologic findings encompassed 28 appendixes with adhesions, 14 with endometriosis, four with focal collections of chronic inflammatory cells and hemosiderin-laden macrophages on the external surface, one benign mucocele, and one carcinoid.

Comment

The lack of complications after incidental appendectomy, as reported in this series and not noted elsewhere, prompts us to recommend laparoscopic appendectomy in qualified hands, an opinion shared by others with similar case experiences. Schreiber,[15] for example, in 1987 reported 151 cases of laparoscopic incidental appendectomy with only one complication, an endocoagulative injury to the cecum that was successfully repaired by subsequent laparotomy. Schreiber, who essentially followed the procedures of Semm[16] except for not "countersinking" the appendiceal stump, considered laparoscopic appendectomy " a worthwhile alternative to classical appendectomy, even in acute appendicitis." Average hospitalization was 8.1 days (range, 4 to 22 days), and the author emphasized the subjective well-being of patients.

Gotz et al.[17] recently reported efforts initiated in May 1989 to make laparoscopic appendectomy for acute or chronic appendicitis a routine tool of the general surgeon. They simplified procedures espoused by Semm,[16] including not invaginating the appendiceal stump, and adopted video monitoring as used and promulgated by Nezhat.[12, 13] On the basis of 388 patients with a variety of acute and chronic vermix disease, they concluded that laparoscopic appendectomy is "a practicable and reasonable alternative to routine surgery." Complications, all successfully treated by subsequent laparotomy and mostly occurring early in the series, prompted the authors to emphasize that there was a significant learning curve and that 1½ years of training was required for proficiency in the procedures. Average hospitalization was 1 week. Most patients resumed a normal diet the day after surgery. Complaints of postoperative ab-

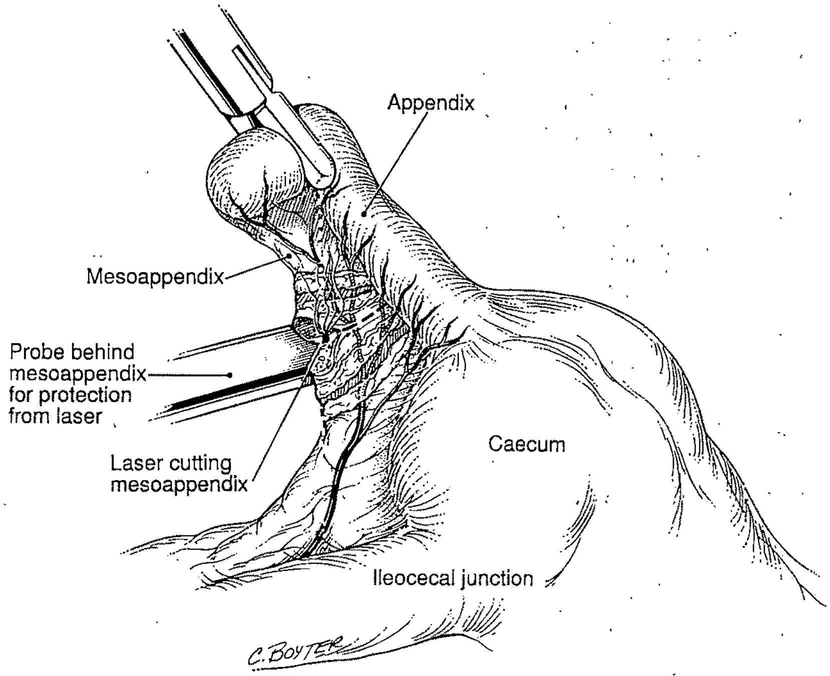
Appendix
Mesoappendix
Probe behind mesoappendix for protection from laser
Laser cutting mesoappendix
Caecum
Ileocecal junction
C. Boyter

Fig. 3. Mesoappendix being cut.

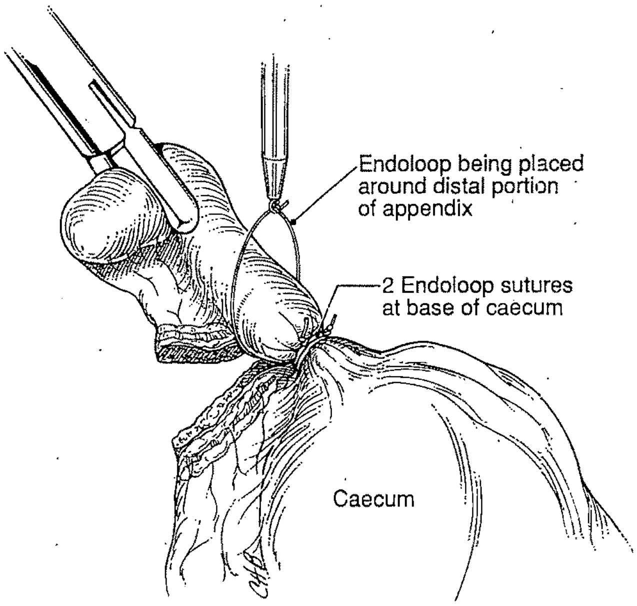
Endoloop being placed around distal portion of appendix
2 Endoloop sutures at base of caecum
Caecum

Fig. 4. Appendix is being doubly sutured and ligated.

dominal pain were seldom made, and the authors regarded this as "certainly responsible for the early mobilization of most patients."

Unlike Semm,[16] we do not bury the appendiceal stump, thus avoiding the risk of invading the lumen of the bowel with a needle. Relevant is a prospective randomized trial reported in 1985 by Engstrom and Fenyo[18] involving 735 consecutive appendectomies in which stump invagination was compared with simple ligation. The authors recommended the latter as the procedure of choice. Also, we do not, as advocated by Semm,[16] ligate the appendiceal artery but rely on bipolar cautery for coagulating the appendiceal artery and mesoappendix. The major difference between our technique and procedures followed by authors previously cited is the use of the carbon dioxide laser for cutting and location of the ancillary trocars.

It is relevant to note here that there is, for reasons not entirely clear, more than a tenfold variation in the published pathology rates associated with incidental appendectomy. Ikard[19] in 1987, for example, in a prospective randomized study to determine infection rates in 100 patients undergoing cholecystectomy, 47 of whom also had incidental appendectomy, found appendiceal pathologic conditions in only 6% of cases. In contrast, in 1983 von Rechenberg[20] reported abnormal appendixes in 82.9% of 113 incidental appendectomies among 627 gynecologic laparotomies. Also, among 5369 gynecologic laparotomies studied by Krone and Sperke,[21] 1718 preventive appendectomies were done; 368 appendixes (21.4%) were normal, 136 (7.9%) revealed acute appendicitis, and 1118 cases (65.1%) showed chronic disease, including an unspecified number with fibroid scarring and total obliteration. The remaining 96 cases (5.6%) showed carcinoid, mucocele, and endometriosis. Interestingly, a report by Westermann et al.[22] in 1986, from a teaching service where

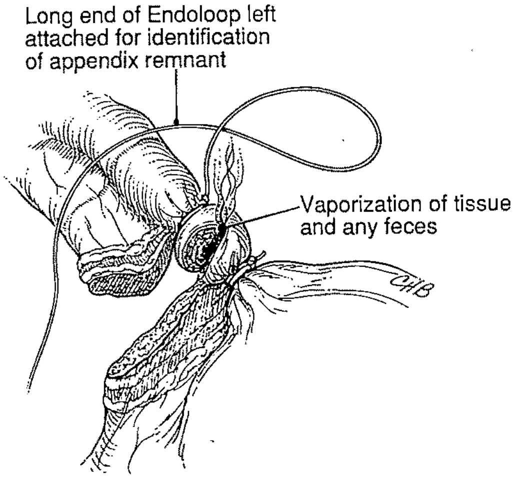
Long end of Endoloop left attached for identification of appendix remnant
Vaporization of tissue and any feces
CHB

Fig. 5. Appendix is being cut and seared with carbon dioxide laser.

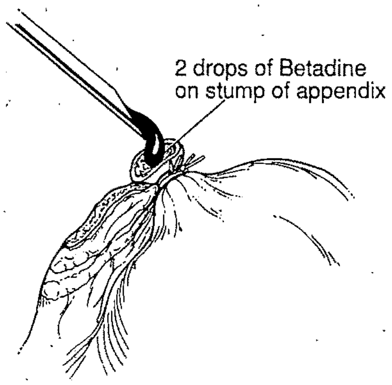

Fig. 6. One or two drops of povidone-iodine is applied to appendiceal stump.

routine appendectomy during extensive gynecologic surgery was the rule, noted histologic abnormalities in 28.7% (68) of 233 cases. The authors chose to view luminal fibrosis, peri-appendicitis (acute and chronic), chronic inflammation, mucosal hyperplasia, and a foreign body reaction found in 33 cases (14.0%) as equivocal or variations of normal but regarded 34 (14.6%) as pathologic. The latter included metastatic carcinoma of the endometrium and ovaries, endometriosis, carcinoma of the cecum, carcinoid, mucocele, and tuberculosis. The authors did, however, note that what fraction of appendiceal abnormalities should be viewed as definitely pathologic was a controversial and unresolved issue.

In view of the reported variables of patient selection and preference, preoperative diagnosis,the character of the surgery, pathologic terminology, age, sex, and sample size, it would hardly be possible to predict appendiceal findings in the group of patients in the current study. All were known to have endometriosis and all were referred to the endometriosis clinic for endoscopic surgery. Our findings of normal appendixes in only half of the cases adds an additional dimension to the literature on incidental appendectomy.

Regarding risk versus benefit, four prospective randomized studies noted that appendectomy, whether termed *elective, incidental, in passing, preventive,* or *prophylactic,* did not significantly alter patient response to major laparotomies. Two sereis concerned cholecystectomy with and without appendectomy.[19-23]

The outcome of elective cesarean section was not significantly influenced by adding appendectomy.[24] Appendectomy was randomly added in patients without intraabdominal injury undergoing exploratory laparotomy for penetrating abdominal trauma.[25] This report viewed incidental appendectomy as a relatively innocuous procedure in trauma patients. These authors view elective or incidental appendectomy as a safe procedure, having minimal risks when appropriately added to major surgery by laparotomy. Their results reinforced our conviction that elective appendectomy as reported in this study adds minimal risk.

Whatever small risk there might be, including that attributable to anesthesia, is far outweighed by benefits gained: first, from eliminating appendectomies as a future hazard, particularly in women of reproductive age; second, from simplifying differential diagnosis involving subsequent, acute intraabdominal and pelvic emergencies; third, from removing pathologic appendixes that escaped prior diagnosis.

In summary, 100 incidental appendectomies, performed during operative laparoscopies carried out to evaluate and treat endometriosis with various gynecologic disorders, added from 4 to 21 minutes to the procedures. Except in one patient with transient fever (101° F), which resolved within 24 hours, and mild periumbilical ecchymosis, there were no intraoperative or postoperative complications of note. All of the patients left the hospital within 24 hours of surgery. Average hospital stay was 14 hours.

Follow-up of all patients for a minimum of 8 months revealed no apparent morbidity or change in recovery time attributable to appendectomy.

The risks are minimal and are outweighed by the benefits of eliminating future emergency appendectomies, simplifying differential diagnoses of pelvic pain, and removing appendiceal abnormality.

We conclude that in the hands of experienced endoscopists, incidental prophylactic or elective appendectomy can be performed safely by videolaseroscopy.

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Use of hydroxyurea in chronic myeloid leukemia during pregnancy: A case report

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The concomitant occurrence of pregnancy and chronic myeloid leukemia is uncommon. The use of hydroxyurea in chronic myeloid leukemia during pregnancy is unknown. We report on a patient with chronic myeloid leukemia in whom hydroxyurea was used during pregnancy with a successful outcome for both mother and fetus. (Am J Obstet Gynecol 1991;165:565-6.)

Key words: Chronic myeloid leukemia, pregnancy, hydroxyurea

Various modalities of treatment have been used in the management of chronic myeloid leukemia during pregnancy, including the use of cytotoxic drugs, splenic irradiation (with shielding of the uterus), and, more recently, leukapheresis.

Hydroxyurea, a cytotoxic agent that inhibits dexoyribonucleic acid synthesis, is useful in both the chronic and the accelerated phases of the course of chronic myeloid leukemia. Its role in the pregnant patient has not been elucidated. In this case report we describe the successful management with hydroxyurea in a case of chronic myeloid leukemia throughout the patient's pregnancy.

Case report

A 33-year-old woman (gravida 3, para 1) with chronic myeloid leukemia was 12 to 14 weeks pregnant at routine follow-up examination. She had two previous pregnancies. The first resulted in a normal vaginal delivery 12 years previously, and the second ended in a miscarriage at about the fourth month of pregnancy, 8 years previously. Chronic myeloid leukemia was diagnosed 7 months previously. On initial examination, she had evidence of mild pallor, splenomegaly (3 cm), and hepatomegaly (2 cm). The white blood cell count was 146 × 10^9/L with 72% neutrophils, 1% promyelocytes, 9% myelocytes, 8% metamyelocytes, 6% lymphocytes, 1% monocytes, and 3% basophils. The hemoglobin level was 11.4 gm/dl and the platelet count was 284 × 10^9/L. Results of the Philadelphia chromosome test were positive.

The pregnancy was discovered at the end of the first trimester, and at that time she was being treated with hydroxyurea. Therefore the drug was continued throughout the pregnancy. A dose ranging between 500 mg and 1 gm orally daily was used (during the pregnancy). The patient's course from diagnosis until death is depicted in Fig. 1. At 36 weeks' gestation she went into spontaneous labor and had a vaginal delivery of a normal, physically healthy 2670 gm male infant with normal blood counts. There were no perinatal complications.

The mother remained well for 16 months after delivery but died 2 months later, after acceleration of the disease. At present, the baby is 26 months old, is physically and developmentally normal, and has a hemoglobin level of 11.9 gm/dl, a white blood cell count of 7.12 × 10^9/L, and a platelet count of 336 × 10^9/L.

Comment

The association of chronic myeloid leukemia and pregnancy is infrequent. The successful management of chronic myeloid leukemia during pregnancy revolves around the need to treat the mother appropriately to ensure and maintain maternal well-being and the desire to avoid fetal compromise, especially in the first trimester of pregnancy.

To this end, accepted treatment modalities in such patients generally involve the use of cytotoxic agents, ionizing radiation, and leukapheresis.

Of the cytotoxic drugs, busulfan has been the most widely used agent with a favorable outcome in the vast majority of patients.[1] Splenic irradiation with shielding of the uterus has been used without obvious teratogenic effects. However, there are potential leukemogenic effects of in utero exposure to radiation. More recently, leukapheresis has been used successfully[2] and may be considered the treatment of choice in selected patients because of its lack of teratogenic effects. It also has the added advantage of not adversely influencing the

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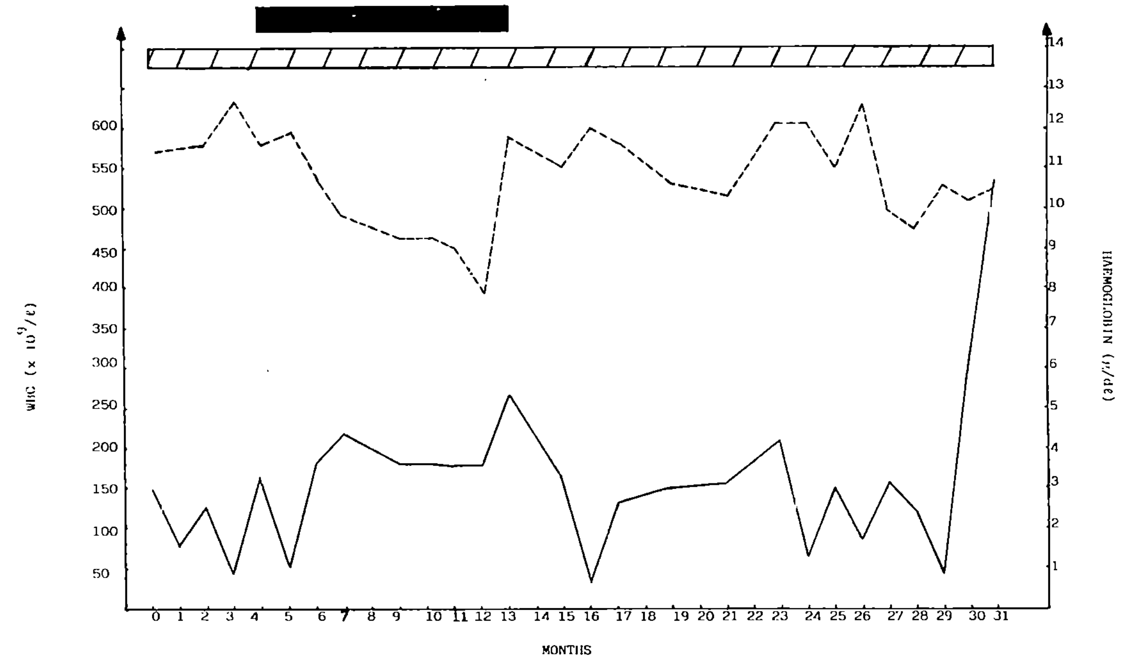

Fig. 1. Summary of patient's course from diagnosis until death. *Solid line,* White blood cell count ($\times 10^9$/L). *Dashed line,* Hemoglobin level (grams per deciliter). ■, Pregnancy. ▨, Treatment with hydroxyurea.

course of chronic myeloid leukemia and can result in improvement in symptoms. However, it is costly and time consuming and may be inconvenient for some patients. In certain centers it may not be readily available. In recent years hydroxyurea has been increasingly used in the management of patients with chronic myeloid leukemia. Its advantages are ease of administration, short duration of action, and absence of cross sensitivity with and resistance to alkylating agents. Compared with those of other cytotoxic drugs, side effects are uncommon. The role of hydroxyurea in the pregnant patient has not been elucidated.

It appears from this case report that hydroxyurea may be well tolerated and serve as a useful alternative to leukapheresis in pregnant patients with chronic myeloid leukemia, in situations where leukapheresis is not available or practicable. Further reports in this group of patients will define the precise role of hydroxyurea in chronic myeloid leukemia during pregnancy.

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Aprotinin blocks the binding of pro atrial natriuretic peptides 1 to 30, 31 to 67, and 99 to 126 to human placental membranes

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Two peptides with natriuretic and diuretic properties consisting of amino acids 1 to 30 and 31 to 67 of the 98 amino acid N-terminal end of the prohormone of atrial natriuretic factor, which normally circulates in humans, were investigated to determine if they have specific binding sites of placental membranes. Competitive binding experiments revealed that atrial natriuretic peptides 1 to 30, 31 to 67, and 99 to 126 (i.e., C-terminus) each had specific and separate binding sites. The dissociation constants for atrial natriuretic peptides 1 to 30, 31 to 67, and 99 to 126 binding to human placental membranes were similar at 4.3 ± 0.6 nmol/L, 3.1 ± 0.4 nmol/L, and 2.9 ± 0.5 nmol/L, respectively. Each peptide bound to the placental membranes between 10^{-8} and 10^{-11} mol/L but could bind to the other peptides' receptors only at supraphysiologic concentrations of 10^{-6} and 10^{-7} mol/L. The protease inhibitor aprotinin (50 μg/ml) blocked the binding of the atrial natriuretic peptides 1 to 30, 31 to 67, and 99 to 126 to their respective receptors. These results suggest that atrial natriuretic peptides 1 to 30 and 31 to 67 do not work through the atrial natriuretic factor receptor but rather have their own separate and distinct receptors on placental membranes and that the protease inhibitor aprotinin antagonizes their respective binding to placental membranes, suggesting that integral membrane proteases may be modulators of atrial natriuretic peptides receptor function. (AM J OBSTET GYNECOL 1991;165:567-73.)

Key words: Atrial natriuretic peptides, receptors, protease inhibition, placenta, aprotinin

Cardiac myocytes contain biologically active peptides with natriuretic, diuretic, and vasodilatory properties.[1-3] In addition to atrial natriuretic factor (ANF) the 126 amino acid ANF prohormone contains two other peptides with vasodilatory, diuretic, and natriuretic properties.[2, 3] These two peptides consisting of amino acid 1 to 30 (i.e., proANF 1 to 30 or long-acting sodium stimulator) and amino acid 31 to 67 (i.e., proANF 31 to 67 or vessel dilator) of the ANF prohormone are equally as effective as ANF (amino acid 99 to 126 of the prohormone) in their vasodilatory[2] and natriuretic and diuretic properties.[3] Recently the 98 amino acid N-terminus of the ANF prohormone containing proANF 1 to 30 and proANF 31 to 67 and the C-terminus (i.e., atrial natriuretic factor) were found to circulate in humans[4-15] and to increase in the circulation with the increase in blood volume of a normal pregnancy.[16] In addition to the whole 10,000 molecular weight N-terminus, a 4000 molecular weight peptide consistent with proANF 31 to 67 from the midportion of the N-terminus also circulates as determined by G-50 Sephadex gel permeation chromatography.[13] The fact that proANF 31 to 67 circulates normally in the plasma as a distinct peptide in addition to the whole N-terminus suggests that proANF 1 to 30 and proANF 69 to 98, which normally are attached to proANF 31 to 67 in the N-terminal polypeptide chain, also may circulate after proANF 31 to 67 has been proteolytically cleaved from the midportion of the N-terminus.[12, 13]

Atrial natriuretic factor (i.e., the C-terminus of the prohormone) binds to placental membranes where it may be involved in fluid and electrolyte homeostasis.[17-19] Since proANF 1 to 30 and 31 to 67 have diuretic and natriuretic properties that are similar to those of ANF,[3] this study was designed to determine if the proANFs 1 to 30 and 31 to 67 have separate and distinct binding sites on placental membranes. When these newly discovered peptide hormones were found to have distinct binding sites on placental membranes, this investigation was extended to determine if aprotinin, a protease inhibitor, could block their binding similar to its previously described ability to antagonize the binding of human chorionic gonadotropin to its receptor on ovarian membranes.[20]

Material and methods

Materials. Aprotinin was purchased from Sigma Chemical Co. (St. Louis). ProANF 1 to 30 labeled with

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iodine 125 (specific activity, 1100 Ci/mmol), ^{125}I-proANF 31-67 (specific activity, 1500 Ci/mmol), and ^{125}I-ANF (specific activity, 2000 Ci/mmol) were labeled by the Iodogen method[21] by Peninsula Laboratories (Belmont, Calif.). To determine the specific activity of these three ligands, 0.2 mmol/L of each peptide was conjugated with 500 µCi of sodium iodide. After purification on high-pressure liquid chromatography, the amount of radioactivity in each fraction was added together and then divided by 0.2 nmol/L to determine the specific activity of each of the respective peptides. Unlabeled human proANF 1 to 30, 31 to 67, and human ANF (amino acid 99-126) also were purchased from Peninsula Laboratories.

Purity of peptides by high-pressure liquid chromatography. Each of these peptides was tested for purity on a liquid chromatograph from Waters Associates, which was composed of a model 680 gradient controller, two model 510 high-pressure liquid chromatography pumps, a model U6K injector, a Novapak C18 (5 µm) cartridge column, and a model 481 LC spectrophotometer. Separation was achieved with a linear gradient of 0% to 60% solvent B over 40 minutes at a flow rate of 1.0 ml/min. Solvent A was 0.1% trifluoroacetic acid and water and solvent B was 0.1% trifluoroacetic acid and acetonitrile. The wavelength monitored was 210 nm. Each of the peptides used in this investigation was pure when tested in this manner.

Preparation of placental membranes. Membrane preparation of a plasma membrane fraction of placenta was by the method of Siegel et al.,[22] which we have used previously to prepare smooth membranes from vasculature.[23] Fresh placentas ($n = 6$) from normal term deliveries were washed several times with phosphate-buffered saline solution, pH 7.4, and the supporting amnion and chorion were removed. The tissue (100 to 150 gm) was finely minced, suspended in a buffer containing 5 mmol/L ethylenediaminetetraacetate, 1 mmol/L Tris hydrochloride, pH 7.4, 2 mmol/L magnesium chloride, 1 mmol/L dithiothreitol, and 0.1 mmol/L phenylmethylsulfonylfluoride at a ratio of 6 ml buffer to 1 ml minced placenta, and stored on ice for 30 minutes to allow cell lysing to occur (all further procedures are at 4° C). The cell suspension was homogenized in a Brinkman Polytron homogenizer at slow speed for 1 minute. Aliquots of 25 ml homogenate were layered over 10 ml 44.5% sucrose and 20 mmol/L Tris hydrochloride, pH 7.4, and centrifuged at 70,000 g for 30 minutes. The membrane layer floating on top of the sucrose was isolated and transferred by a pipette into an ultracentrifuge tube containing 0.25 mmol/L sucrose, 50 mmol/L Tris hydrochloride, pH 7.4, 1 mmol/L dithiothreitol, and 0.1 mmol/L phenylmethylsulfonylfluoride. This solution was centrifuged at 40,000 g for 15 minutes, the supernatants from all tubes were discarded, and the pellets were pooled. The pellets were resuspended in 0.25 mol/L sucrose, 50 mmol/L Tris hydrochloride, pH 7.4, 1 mmol/L dithiothreitol, and 0.1 mmol/L phenylmethylsulfonylfluoride. The protein concentration was determined by the Bradford assay[24] with bovine serum albumin used as the standard. Membranes were either used fresh or stored in liquid nitrogen for later use. No loss in receptor activity occurred under these conditions. The use of these human placental membranes was approved by the Human Use Committee for the University of Arkansas for Medical Sciences.

Binding of ^{125}I-labeled proANF 1 to 30, 31 to 67, and 99 to 126. Purified placental membranes were diluted 1:10 in phosphate-buffered saline solution and then incubated with 1 nmol/L ^{125}I-labeled human proANF 1 to 30, 31 to 67, and 99 to 126 (approximately 10,000 counts/min), respectively, in separate polypropylene tubes. Each tube had 100 µl total volume of Hanks' balanced salt solution, pH 7.4, containing 0.1% bovine serum albumin (binding medium). They were incubated at 24° C for 60 minutes. After their respective 1-hour incubations the supernatants were aspirated, the membranes were washed three times with ice-cold phosphate-buffered saline solution containing 0.1% bovine serum albumin, and the membrane-bound radioactivity was measured in a γ-spectrometer. Specific binding was defined as the total binding minus the nonspecific binding in the presence of varying concentrations from 10 pmol/L to 1 µmol/L of the unlabeled atrial natriuretic prohormone peptides. The specific binding for the three ligands at their respective 1 nmol/L concentrations were: proANF 1 to 30, 66%; proANF 31 to 67, 57%; and ANF 99 to 126, 47%.

Analysis of receptor binding data. The receptor binding data were analyzed with the All Fit Dose Response Analysis Program, written in Applesoft Basic by Martin H. Teicher, MD, PhD. This computer program is modified from the original AllFit program of DeLean et al.[25]

Results

Binding of ^{125}I-proANF 1 to 30, ^{125}I-proANF 31 to 67, and ^{125}I-ANF to placental membranes. ^{125}I-proANF 1 to 30, ^{125}I-proANF 31 to 67, and ^{125}I-ANF (99 to 126) were found to bind specifically to placental membranes (Figs. 1 to 3). Unlabeled proANF 1 to 30 displaced ^{125}I-labeled proANF 1 to 30 binding in a concentration-dependent manner on placental membranes (Fig. 1). ANF and proANF 31 to 67 inhibited ^{125}I-labeled proANF 1 to 30 binding at concentrations somewhat above which these peptides are known to circulate, i.e., 10^{-6} mol/L for ANF and 10^{-7} mol/L for proANF 31 to 67 (Fig. 1). Similarly corticotropin, growth hormone, and insulin could displace ^{125}I-proANF 1 to 30 binding

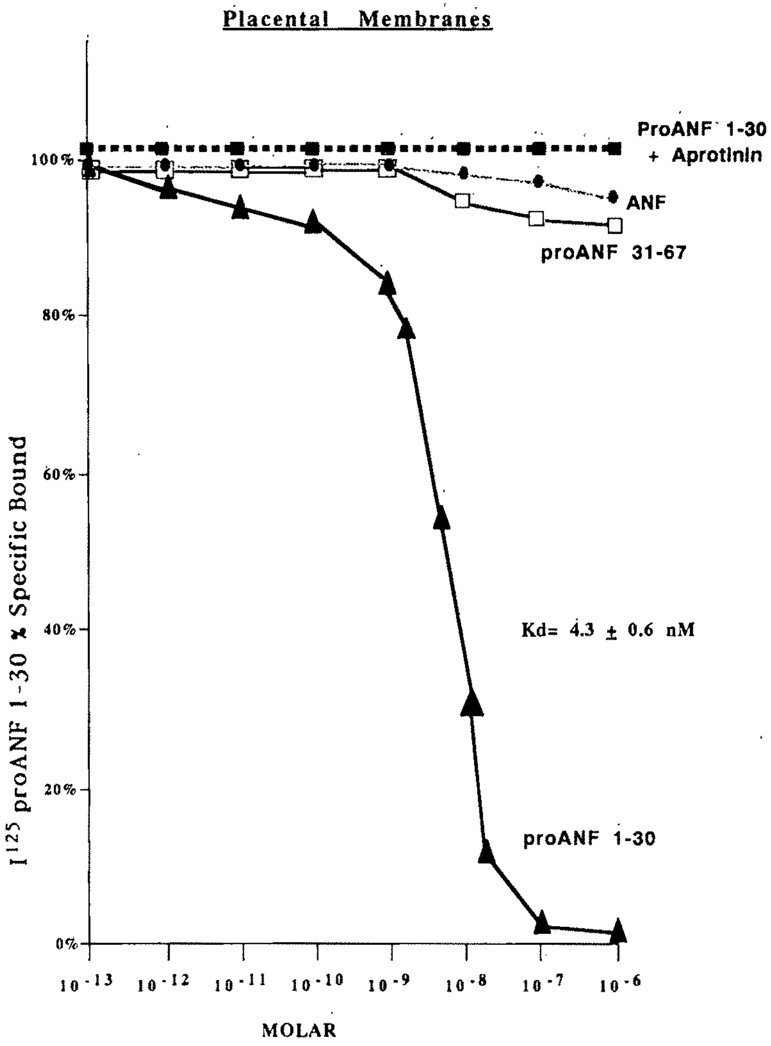

Fig. 1. Concentration-dependent relationship of proANF 1 to 30 binding on placental membranes. ^{125}I-proANF 1 to 30 binding to human placental membranes incubated at 24° C for 60 minutes was inhibited by unlabeled proANF 1 to 30 in a concentration-dependent manner but not by proANF 31 to 67 or ANF. Addition of aprotinin (50 μg/ml) antagonized proANF 1 to 30 specific binding. K_d, dissociation constant.

only at 10^{-4} mol/L concentrations but not at their circulating concentrations (data not shown). Scatchard analysis of the proANF 1 to 30 binding to placental membranes resulted in a straight line, suggesting that these membranes contain a single class of high-affinity binding sites for proANF 1 to 30 with an equilibrium dissociation constant of 4.3 ± 0.6 nmol/L.

The binding of ^{125}I-proANF 31 to 67 to placental membranes was competitively inhibited by unlabeled proANF 31 to 67 between 10^{-9} and 10^{-11} mol/L (Fig. 2). The binding of this peptide hormone could be inhibited by concentrations (10^{-4} to 10^{-7} mol/L) of ANF, proANF 1 to 30, insulin, growth hormone, and corticotropin that are far in excess of their respective circulating concentrations (Fig. 2). Scatchard analysis of the proANF 31 to 67 binding data resulted in a straight line, suggesting that placental membranes contain a single class of high-affinity binding sites for proANF 31 to 67 with an equilibrium dissociation constant of 3.1 ± 0.4 nmol/L.

^{125}I-ANF (99 to 126) also bound to placental membranes (Fig. 3), and its binding was competitively displaced by 10^{-8} to 10^{-10} mol/L of unlabeled ANF but not by proANF 1 to 30, proANF 31 to 67, insulin, growth hormone, or corticotropin in this concentration range (Fig. 3). Scatchard analysis of the ANF binding data revealed a straight line with an equilibrium dissociation constant of 4.9 ± 0.8 nmol/L, indicating a high-affinity binding site for ANF on human placental membranes.

Binding of the three radiolabeled peptides was observed as early as 5 minutes and reached equilibrium at 30 to 40 minutes at 24° C. After 3 hours of incubation at 24° C the amount of specific binding began to de-

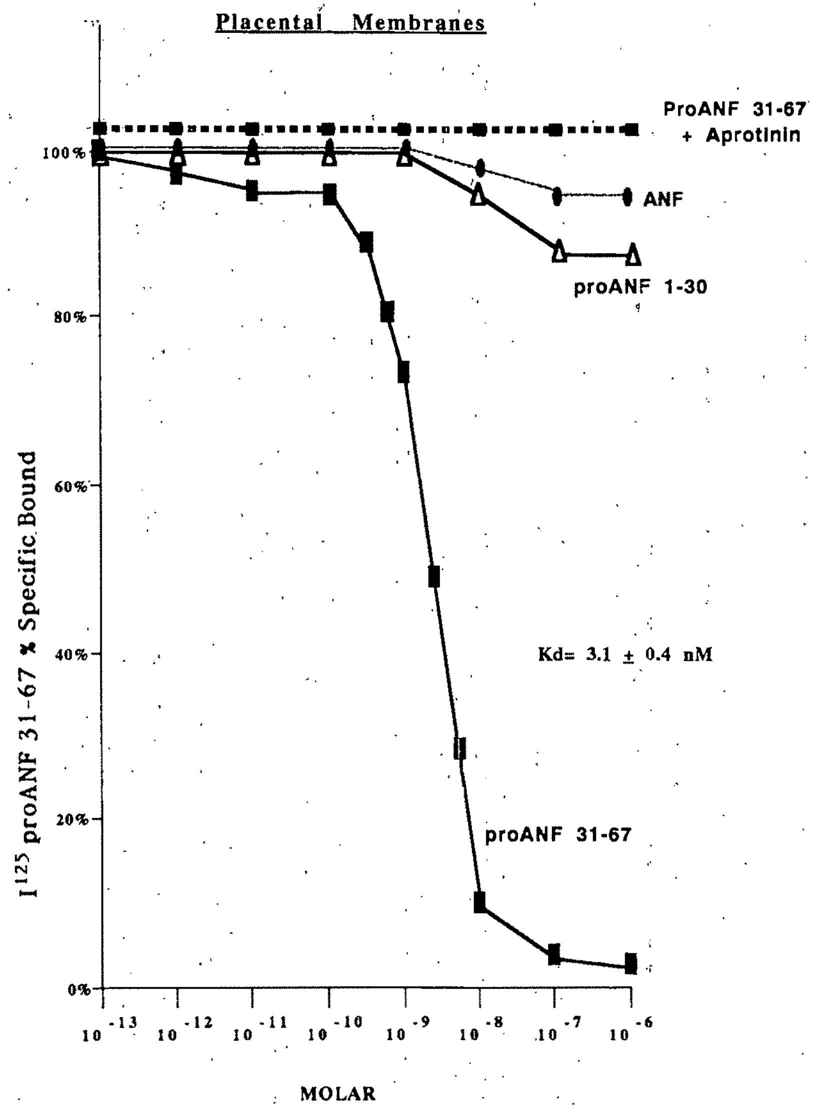

Fig. 2. Competitive binding of ^{125}I-proANF 31 to 67 to placental membranes. ^{125}I-proANF 31 to 67 binding to placental membranes for 60 minutes at 24° C was inhibited by unlabeled proANF 31 to 67 in a concentration-dependent manner but not by proANF 1 to 30 or ANF. Aprotinin (50 µg/ml) antagonized proANF 31 to 67 binding to these placental membranes. K_d, Dissociation constant.

crease. At 4 hours the specific binding of all three peptides had decreased to 50% of that seen at 2 hours. This rapid and reversible binding was also temperature dependent. At 0° C maximal binding was not reached until 60 minutes and did not decrease for up to 4 hours.

Aprotinin (50 µg/ml) blocked the binding of ^{125}I-proANF 1 to 30 (Fig. 1), ^{125}I-proANF 31 to 67 (Fig. 2), and ^{125}I-ANF (Fig. 3), respectively, to placental membranes at 24° C. This reduction of ^{125}I-proANF 1 to 30, ^{125}I-proANF 31 to 67, and ^{125}I-ANF binding was temperature dependent, with a maximal inhibition of the binding of each of these peptides at 4° C with 10 µg/ml of aprotinin, whereas at 24° C 50 µg/ml of aprotinin was necessary for maximal inhibition of binding.

Comment

High-affinity membrane binding sites for ANF (amino acid 99 to 126 of the 126 amino acid ANF prohormone) have been characterized previously in placental membranes.[17-19] The present investigation reveals that proANF 1 to 30 (long-acting sodium stimulator) and proANF 31 to 67 (vessel dilator) each have separate and specific binding sites that are distinct from the ANF binding site. The association of each of their respective binding sites was time and temperature dependent. The binding affinities of proANF 1 to 30, proANF 31 to 67, and ANF on placental membranes were observed near their respective circulating concentrations,[4-16] suggesting that these binding sites may be

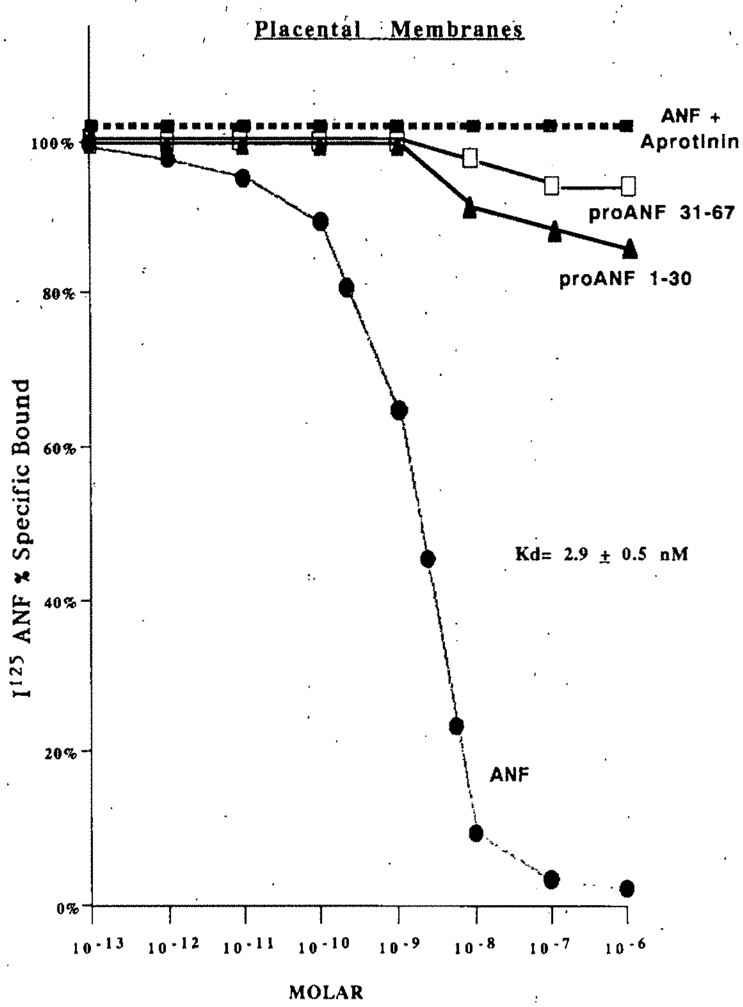

Fig. 3. Competitive binding of ^{125}I-ANF (amino acid 99 to 126 or prohormone) to placental membranes. Placental membranes were incubated at 24° C for 60 minutes with ^{125}I-ANF and increasing concentrations of unlabeled proANF 31 to 67, proANF 1 to 30, or ANF. Aprotinin (50 μg/ml) added to increasing concentrations of ANF antagonized the specific binding of ANF on these placental membranes. K_d, Dissociation constant.

important in mediating fluid and electrolyte homeostasis in human gestation. A study by Hatjis and Grogan[18] indicating that ^{125}I-ANF binds to the placental membrane fraction closer to the fetal circulation than to the maternal circulation is very interesting in this regard since it suggests that certain placental functions may be influenced primarily by the fetal hormonal milieu. ANF levels have been shown to be higher in umbilical arterial plasma than in venous umbilical or maternal plasma, implying fetal synthesis of atrial natriuretic peptides.[26] The possibility that the placenta itself is synthesizing the ANF prohormone awaits further investigation with ANF messenger ribonucleic acid probes of cloned complementary deoxyribonucleic acid. The finding of this investigation that the natriuretic and diuretic peptides proANF 1 to 30, proANF 31 to 67, and ANF have distinct binding sites with nearly equal affinity in placental tissue suggests that proANF 1 to 30, proANF 31 to 67, and ANF may be important in the regulation of fluid and electrolyte transport between fetal and maternal compartments. ProANF 1 to 30 and proANF 31 to 67 are equally potent in comparison with ANF in animal studies in causing a diuresis and excretion of sodium,[3] but the nearly equal binding affinity of each in the placenta cannot be equated with each of the peptides having equal effects on placental homeostasis. The contribution of each of these newly discovered peptides to placental fluid homeostasis and the origin of these peptides in the placenta, whether from maternal, fetal, or placental production, awaits further investigation.

Aprotinin, a protease inhibitor that is widely used in radioimmunoassay systems to prevent proteolysis, clearly antagonized the binding of ^{125}I-proANF 1 to 30, ^{125}I-proANF 31 to 67, and ^{125}I-ANF to placental membranes. Aprotinin previously has been demonstrated to inhibit the binding of human chorionic gonadotropin to its receptor in ovarian plasma membranes.[20] Another protease inhibitor, dansyl-arginyl-(4′-ethyl)piperidine amide, likewise has been shown to inhibit human chorionic gonadotropin binding to its receptor.[27] The exact mechanism of the ability of protease inhibitors to inhibit binding of human chorionic gonadotropin, proANF 1 to 30, proANF 31 to 67, and ANF to plasma membranes is unknown. Since these plasma membranes appear to be rich in proteases,[28] however, it may well be that aprotinin antagonizes the binding of these substances by binding to a protease closely associated with the receptor molecule, as suggested by Wilkes and Hui[20] for the human chorionic gonadotropin receptor, and thereby sterically hinders the entry of these peptides into their binding site on placental membranes. This possible mechanism of action of aprotinin on proANF 1 to 30, proANF 31 to 67, and ANF binding is consistent with the emerging concept that integral membrane proteases are modulators of receptor function.

In conclusion, the presence of specific binding sites for proANF 1 to 30, proANF 31 to 67, and ANF in human placenta is of interest in that it suggests that these three newly discovered peptide hormones may be important in placental hemodynamics, fluid shifts, and electrolyte transport, because the first step in the mechanism of action of most polypeptide hormones is the binding to a receptor in their target tissues. The ability of aprotinin to antagonize the binding of these peptide hormones to placental membranes suggests that integral membrane proteases may be modulators of the receptor function of proANF 1 to 30 (long-acting sodium stimulator), proANF 31 to 67 (vessel dilator), and ANF.

We thank M. Theresa Newton for her excellent technical assistance.

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Hexamethylmelamine chemotherapy for persistent or recurrent epithelial ovarian cancer

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The purpose of this study was to determine the activity and toxicity of hexamethylmelamine chemotherapy in patients with persistent or recurrent epithelial ovarian cancer. Forty-nine women received hexamethylmelamine 100 to 150 mg/day for 14 days, repeated at 4-week intervals. All patients had previously received at least one chemotherapy regimen, and 46 (94%) had received cisplatin. Among 25 patients with clinically measurable disease there were three complete and two partial responses, for an objective response rate of 20%. The mean progression-free interval for responders was 38.6 months versus 9.6 months for nonresponders or patients with nonmeasurable disease ($p < 0.001$). Thirteen patients are alive, eight with no clinical evidence for disease. Only four patients discontinued therapy because of toxic reactions. Hexamethylmelamine appears to be a well-tolerated drug with activity against ovarian cancer previously treated with cisplatin. (Am J Obstet Gynecol 1991;165:573-6.)

Key words: Ovarian cancer, drug therapy, hexamethylmelamine

In spite of the discovery of cisplatin and its analogs and the development of multiagent chemotherapy regimens for the treatment of epithelial ovarian cancer, fewer than one fourth of women initially seen with stage IIIC to IV disease will ultimately survive cancer. Recurrent ovarian cancer is often drug or radiation resistant and incurable, even with a low tumor burden. There is no standard therapy for persistent or recurrent ovarian cancer that can be considered curative in a majority of cases. Second- and third-line treatments are frequently handicapped by a performance status decreased from advanced disease.

Hexamethylmelamine was introduced into clinical cancer trials in 1964. The mechanism of action of hexamethylmelamine is unknown, although it is structurally similar to the alkylating agent triethylenemelamine and probably does not involve alkylation. Early experiences with single-agent hexamethylmelamine consistently demonstrated activity in epithelial ovarian cancer, with response rates of 15% to 20% in previously treated patients and >30% in patients new to chemotherapy.[1, 2] Hexamethylmelamine is attractive as an anticancer drug because myelosuppression is generally not the dose-limiting toxicity. Nonetheless, a role for hexamethylmelamine in ovarian cancer treatment remains undefined because of the relative lack of large, adequately controlled comparative studies.[3]

We have used hexamethylmelamine for >8 years in the treatment of ovarian adenocarcinoma. The purpose

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of this report is to review our experience with this drug in patients with recurrent or persistent ovarian cancer previously treated with cisplatin-based chemotherapy.

Material and methods

Sixty-two patients with recurrent or persistent epithelial ovarian cancer were treated with hexamethylmelamine chemotherapy at the gynecologic oncology service at the University of North Carolina Hospitals between April 1, 1982, and Oct. 31, 1989. Patients were identified by reviewing chemotherapy clinic and disposition conference records. The disposition conference is a weekly multidisciplinary meeting to discuss the evaluation and treatment of all patients with gynecologic malignancies.

Hexamethylmelamine capsules were administered at a dose of 100 to 150 mg/day for 14 days, with courses repeated at 4-week intervals until cancer progression or toxic reaction. Doses were not adjusted according to body surface area calculations. An adequate trial of therapy was defined as the completion of one course of treatment and a follow-up examination. Six patients were excluded from analysis either because they never took the drug ($n = 2$) or they never returned for follow-up ($n = 4$). Additionally, seven women were excluded because hexamethylmelamine was given in combination with other chemotherapy drugs ($n = 2$), recurrent or persistent ovarian cancer was not documented ($n = 1$), or the nature of the primary tumor was uncertain ($n = 4$). Therefore 49 patients formed the cohort for analysis.

Medical records were analyzed to determine survival, the frequency of objective responses (including progression-free interval and response duration), and treatment side effects. Determining objective response rates depended on measurable disease, functionally defined through the ability to measure cancer in two perpendicular diameters by physical examination or radiologic studies. Response criteria included complete response, defined as the disappearance of all measurable disease; partial response, defined as a >50% decrease in the product of perpendicular diameters for measurable disease; stable disease, defined as no change in tumor measurements; and progression, defined as a >50% increase in the product of perpendicular diameters or the appearance of new lesions. A persistently elevated serum CA 125 (>35 U/ml) was considered indicative of disease persistence. Twenty-five women in this series had measurable disease, and 24 had nonmeasurable disease. Patients with nonmeasurable disease were included in survival and toxicity analyses, but were ineligible for response evaluation. All subjects were followed for a minimum of 12 months, except for those who died earlier of cancer. Women with adequate trials of therapy who subsequently were lost to follow-up were presumed dead of disease. Survival statistics were calculated from the initiation of hexamethylmelamine chemotherapy.

Statistical analyses of differences in sample means used a two-sided Student t test. Significance was defined at $p < 0.05$.

Results

The mean age of the 49 patients was 58.8 years (range 26 to 79 years). Eighty-two percent (40/49) of patients had stage III to IV disease when ovarian cancer was first diagnosed, and 93% had grade 2 to 3 tumors. There were no significant differences between patients with measurable versus nonmeasurable disease with respect to age, stage, or tumor grade.

All patients had persistent or recurrent epithelial ovarian cancer and previously had received a minimum of one chemotherapy regimen. Fourteen women had received two, and four women had received 3 prior chemotherapy regimens. Cisplatin-based chemotherapy was previously administered to 94% (46/49) of patients. Sixteen women previously received radiation therapy, including intraperitoneal radioactive chromic phosphate ($n = 12$), pelvic radiation ($n = 2$), or whole abdominal radiation ($n = 1$). One patient received both pelvic radiation therapy and intraperitoneal radioactive chromic phosphate.

Seventy-five percent (18/24) of the women with nonmeasurable disease started hexamethylmelamine chemotherapy because of persistent disease documented at surgical reexploration. Six women started hexamethylmelamine chemotherapy because of inability to complete the initially prescribed therapy, persistent CA 125 elevation after completion of primary therapy, suspicious findings on physical examination or on radiologic study that were not measurable, or refusal to undergo operative reassessment with previous advanced ovarian cancer and large residual tumor volume after surgical debulking. Among the 25 patients with measurable disease, 11 (44%) had tumor masses >5 cm in diameter and 7 (28%) had masses >10 cm in size. The difference in the mean number of hexamethylmelamine courses completed for subjects with measurable (9.0 ± 8.6) versus nonmeasurable (9.6 ± 6.8) disease was not significant.

Seven women with nonmeasurable disease are alive 10 to 35 months (mean 21.3 ± 8.1 months) after the initiation of hexamethylmelamine chemotherapy, and five remain clinically without evidence of disease.

There were three complete and two partial responses among patients with measurable disease, for an objective response rate of 20%. Each objective response occurred in a patient previously treated with cisplatin. All three complete responders remain in clinical remission. One patient with a partial response died at 24 months, and the other is alive with disease at 27 months. Two women currently undergoing treatment at 7 and 28 months are alive with stable disease. The mean progression-free interval for patients with objective responses is 38.6 months (range 14 to 97 months), and the mean progression-free interval for nonresponders plus patients with nonmeasurable disease is 9.6 months (range 1 to 35 months); this difference is significant ($p < 0.001$). No patient whose disease progressed during cisplatin-based chemotherapy responded to hexamethylmelamine.

Nonhematologic toxic reactions to hexamethylmelamine chemotherapy were mild (Gynecologic Oncology Group grade 0 to 1) and generally consisted of either acral paresthesias ($n = 10$) or nausea ($n = 5$). Other complications of treatment included weakness ($n = 3$), myalgias ($n = 2$); and zoster infections ($n = 2$). Myelosuppression was infrequent; three patients had nadir leukocyte counts of $<3000/\mu l$, and no patient's leukocyte count dropped to $<2000/\mu l$. One patient had a nadir platelet count of $94,000/\mu l$. Four patients stopped treatment because of symptoms unrelated to disease progression: nausea, vomiting, and abdominal cramping ($n = 3$); dermatitis ($n = 1$).

Comment

Until it is possible to reliably diagnose ovarian cancer at an early stage, oncologists will continue to face the dilemma of advanced, large-volume disease. Clinical response rates to cisplatin-based multiagent chemotherapy are high, although only 20% to 30% of patients will prove to be in complete pathologic remission at the time of surgical reassessment.[4,5] Disease will recur in as many as 50% of patients in spite of negative findings at second-look laparotomy.[6] Chronic dose-limiting toxic reactions to cisplatin (neurotoxicity, nephrotoxicity, anemia) preclude its use for protracted treatment and indicate the need for alternative therapies.

The activity of hexamethylmelamine in epithelial ovarian cancer was firmly established by Wharton et al.,[2] who reported their series of 54 women treated at the M. D. Anderson Hospital. All patients had stage III to IV ovarian adenocarcinoma, and none had received prior radiation or chemotherapy. Objective responses occurred in 31.5% of patients, with eight complete clinical responses. The test results of the gross specimens of 5 patients were negative for persistent disease at surgical reassessment, and the results of all biopsies were negative in 3 cases. The median disease-free interval was 8 months, and recurrent cancer developed in 70% (12/17) of patients. The results with hexamethylmelamine are similar to results with alkylating agent chemotherapy under similar clinical circumstances.

After surgical staging and tumor debulking, combination chemotherapy is the mainstay of ovarian cancer treatment. Many chemotherapy regimens use cisplatin along with an alkylating agent; therefore second-line therapy of persistent or recurrent ovarian cancer must not be cross-resistant to these agents.

Johnson et al.[7] reported an objective response rate of 28% in a series of 21 women treated with hexamethylmelamine chemotherapy. There were no complete responders. All patients received hexamethylmelamine after manifesting progressive disease while receiving an alkylating agent. Dose modifications were necessary in 90% of patients because of toxic reactions, most commonly nausea and vomiting. Several other investigators have demonstrated the activity of hexamethylmelamine chemotherapy in ovarian cancer resistant to alkylating agents with response rates of 16% to 21%.[8-10] These studies were consistent in the frequency of objective responses, the infrequency of complete responses, and the lack of myelosuppression as a dose-limiting toxicity. Although response rates were modest, some ovarian cancers refractory to alkylating agents evidently are not cross-resistant to hexamethylmelamine.

Response rates achieved with hexamethylmelamine chemotherapy for patients previously treated with cisplatin are more varied. Stehman et al.[11] had no objective responders in their series of 18 patients and concluded that hexamethylmelamine has insignificant activity in cisplatin failures. Rosen et al.[12] contradicted this conclusion when they reported a 25% objective response rate—including four complete responses—among 20 evaluable patients treated with hexamethylmelamine chemotherapy.

Recently, Manetta et al.[13] reported their results for 52 women with advanced ovarian cancer. Cisplatin and cyclophosphamide (with or without doxorubicin) were previously used in 92% of cases. Eight patients (15%) had no evidence of disease after hexamethylmelamine chemotherapy, and five women remained in complete clinical remission through 15 to 65 months of follow-up. Unfortunately, 87.5% of patients categorized as responders had nonmeasurable disease.

The 20% objective response rate to hexamethylmelamine chemotherapy in our series of extensively treated patients is noteworthy. All patients who responded to hexamethylmelamine had previously received cisplatin and had measurable disease. Eight women remain clinically without evidence for disease.

The common dose-limiting toxicities of hexamethylmelamine are gastrointestinal, peripheral neuropathy, and myelosuppression. Most investigators have used hexamethylmelamine doses of 240 to 350 $mg/m^2/day$; dose reductions, treatment delays, or discontinuations were necessary in up to 50% of patients.[1] We chose lower doses of hexamethylmelamine because of our extensively pretreated patient population. The virtual absence of severe (Gynecologic Oncology Group grade 3 or 4) toxic reactions suggests that higher hexamethylmelamine doses may be appropriate.

Since its introduction >25 years ago, a role for hexamethylmelamine in the treatment of epithelial ovarian cancer remains in question. We believe that through the development of well-designed prospective clinical trials an answer can and should be achieved.

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Fetal breathing movements before oxytocin induction in prolonged pregnancies

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Fetal breathing movements were recorded before elective labor induction in 65 patients with uncomplicated prolonged pregnancies. Breathing activity was observed in 41 patients and was absent in 24. Comparison of these two groups indicated that the induction period was significantly shorter and the oxytocin requirement significantly lower in the group with no fetal breathing movements (mean, 319.3 vs 648.5 minutes and 1133.7 vs 2708.4 mIU, respectively). These findings remained valid regardless of Bishop scores. The data suggest that fetal breathing movements are predictive of the effectiveness of oxytocin induction in prolonged pregnancies. (AM J OBSTET GYNECOL 1991;165:577-81.)

Key words: Fetal breathing movements, labor induction, prolonged pregnancy

The optimal management of postterm pregnancy is as yet unestablished, and probably no obstetric disorder is surrounded by more confusion that postdatism.

Physiological placental aging causes progressive uteroplacental insufficiency, which in turn leads to increased rates of fetal asphyxia, oligohydramnios, and in severe cases, nervous system damage and death.[1] The decreased placental blood flow in postdate pregnancies represents a continuum that may in fact begin before 42 weeks' gestation. Studies suggest that perinatal morbidity increases after 40 weeks' gestation.[2, 3]

In uncomplicated, well-established, postterm pregnancies, induction of labor is generally recommended if the cervical score is favorable; however, the proper procedure in cases of unripe cervix remains controversial.[4, 5]

Fetal breathing movements are an integral part of normal physiological development, beginning early in gestation. Significant increase of the incidence of fetal breathing movements by maternal injection of glucose in term pregnancies was previously reported.[6] Studies have shown that fetal breathing activity gradually begins to diminish at 72 hours before labor and ceases completely during the active phase of labor.[7-9] The increase in the level of prostaglandins during labor may exert a dual and simultaneous influence, activating myometrial contractility[10] and inhibiting the fetal respiratory center.[11, 12] Decreased fetal breathing movements have been shown to be a sensitive parameter in distinguishing between true and false premature labor[13-15] and in the diagnosis of true term labor.[16]

The aim of this study was to investigate fetal breathing movements as a possible parameter for the prediction of the effectiveness of oxytocin induction in postdate pregnancies.

Patients and methods

The study group consisted of 65 patients at gestational age 287 to 294 days who underwent fetal testing before the induction of labor at the Assaf Harofe Medical Center between June 1, 1988, and June 1, 1989.

To assure the accurate assignment of gestational age, the study patients were required to meet the following criteria: a reliable last menstrual period (known date); a history of regular menstrual cycles; and one first-trimester or two second-trimester ultrasonographic examinations confirming the menstrual age.

Patients with pregnancy-induced hypertension, diabetes mellitus, previous cesarean section, intrauterine growth retardation, approximate fetal weight >4300 gm, or malpresentation were excluded from the study.

Patients did not take any medication except iron or vitamin supplementation for at least 2 weeks before admission, fasted for at least 8 hours, and did not smoke during the study.

All studies began between 8 and 9 AM when an intravenous infusion of 500 ml 10% glucose was started and administered over a 1-hour period. Nonstress tests were carried out throughout the infusion. Those with reactive NST results were examined for fetal breathing movements within 10 minutes from completion of the glucose infusion, using real-time ultrasonography. Fetal breathing was considered to be present when it was sustained ≥20 seconds, and absent when no sustained movement could be detected within a 45-minute pe-

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Table I. Characteristics of patient population

Patient data	*FBM+*	*FBM−*	*p Value**
No. of patients	41	24	—
Age (yr) (mean ± SEM)	27.2 ± 2.4	26.6 + 2.6	NS
Primipara/multipara	12/29	7/17	NS
Gestational age (days)	292.2 ± 2.1	290.1 + 1.9	NS
Bishop score 3-6/>6	28/13	10/14	<0.03

FBM+, Presence of fetal breathing movements; *FBM−*, absence of fetal breathing movements.

*The two-tailed Student *t* test for independent samples.

riod. Results were sealed in an envelope and revealed only at the termination of the entire study.

Patients were then transferred to the delivery unit for cervical scoring with the method of Bishop.[17] Scores ranged from very low (highly unfavorable cervix) (0 to 2), to low (3 to 5), to high (6 to 10). All scores were recorded by the same senior author (N.B-N.). Patients with highly unfavorable cervices were eliminated from the study and treated either expectantly or by intracervical prostaglandin E_2 gel application. Patients were divided retrospectively into two groups according to the presence or absence of fetal breathing movements. Patient data are shown in Table I.

Oxytocin induction was conducted in three stages:

1. Infusion was begun at a rate of 2 mIU/minute and increased by 1 mIU/minute at 30-minute intervals until three contractions per 10 minutes were achieved.
2. This was followed by a latent phase, during which regular uterine contractions began. When the cervix was effaced and dilated 2 to 3 cm, the membranes were artifically ruptured, internal cardiotocography was initiated, and continuous lumbar epidural block was begun.
3. During the final (active) phase, oxytocin was titrated in accordance with intrauterine pressure measurements to 250 to 300 Montevideo Units. Full dilatation of the cervix was considered as the end point of the study. Cardiotocography was continued throughout until delivery. Scalp pH determinations were conducted in cases of suspected monitored fetal distress.

In all patients induction and delivery were performed under the supervision of the same author (N.B-N.).

Results

Of the 65 patients, 41 showed fetal breathing movements and 24 had no fetal breathing movements. More than 90% of the patients in the latter group were delivered of infants spontaneously, compared with 78% of those with breathing activity. Cesarean sections were performed in four patients, all in the group with fetal breathing movements (one with a high Bishop score and 3 with low scores); indications were fetal distress in one case and combined dysfunctional disorders in three cases. Mean fetal weight (3608 and 3728 gm) and incidence of macrosomia (15% and 17%) were similar in both study groups (Table II).

The data concerning time and quantity of oxytocin infused from the onset of induction to the end of the first stage of labor do not include the four patients delivered by cesarean section.

The mean time required to achieve three uterine contractions per 10 minutes was significantly longer in the group with fetal breathing movements (129.6 minutes) than in the patients who showed no fetal breathing movements (72.2 minutes) (Table III). The mean durations of the latent and active phases were 3 hours and approximately 1.5 hours longer, respectively, in the group with fetal breathing movements. The mean oxytocin requirement from onset of induction to the end of the first stage of labor was more than twofold in the group with fetal breathing movements compared with the group without fetal breathing movements (2708.4 and 1133.7 mIU, respectively).

When the patients were differentiated according to Bishop scores, results showed 34 patients with low scores (3 to 5) and 27 with high scores (6 to 10).

Of the patients with low Bishop scores, 24 (>70%) showed fetal breathing movements and 10 did not. Comparison of these subgroups indicated a significantly longer mean induction time (785.2 vs 369.0 minutes) and a significantly higher mean total oxytocin requirement (3348.4 vs 1418.0 mIU) for the patients with fetal breathing movements (Table IV).

Of the 27 patients with high Bishop scores, 13 showed fetal breathing movement and 14 did not. The former subgroup required a significantly longer induction period (396.1 vs 283.9 minutes) and significantly more oxytocin to induce labor (1526.9 vs 930.7 mIU) than the patients without fetal breathing movements (Table V).

Patients with low Bishop scores and no fetal breathing movements reached the end of the first stage of labor after a mean period of 6 hours and 10 minutes of oxytocin infusion, compared with 6 hours and 40 minutes in patients with high Bishop scores and fetal breathing activity. The mean oxytocin requirement was similar in both groups (3.8 and 3.9 mIU/minute).

Comment

Although earlier studies have reported an approximate twofold increase in neonatal mortality associated with postdatism,[18] more recent data suggest that the risk is not clearly increased.[19] The improved outcomes seem to be a result of closer fetal surveillance in postterm pregnancies. In a review of antepartum testing in

Table II. Mode of delivery and neonatal outcome of total patient population

	FBM+ (N = 41)		*FBM− (N = 24)*		
	n	*%*	*n*	*%*	*p Value**
Mode of delivery					
Spontaneous	32	78.0	22	91.6	NS
Cesarean section	4	9.7	0	0	NS
Instrumental	5	12.2	2	8.3	NS
Fetal outcome					
Weight (gm, mean ± SEM)	3608.3 ± 671.2		3718.6 + 710.1		NS
Apgar score <7 (5 minutes)	1	2.4	0	0	NS
Macrosomia (>4000 gm)	6	14.6	4	16.6	NS

Abbreviations same as in Table I.
*The two-tailed Student *t* test for independent samples.

Table III. Length of induction and oxytocin requirement in patients with and without fetal breathing movements

	FBM+ (n = 34)	*FBM− (n = 27)*	*p Value**
Induction period (minutes):			
Time to 3 contractions/10 minutes	129.6 ± 90.8	72.2 ± 47.1	<0.01
Latent phase	356.2 ± 253.2	167.3 ± 88.2	<0.001
Active phase	167.2 ± 89.5	79.8 ± 39.6	<0.001
Total induction time	648.5 ± 354.0	319.3 ± 137.0	<0.001
Oxytocin requirement (mIU)			
Oxytocin to 3 contractions/10 minutes	478.9 ± 411.1	221.6 ± 163.0	<0.01
Latent phase	1546.3 ± 1368.6	632.1 ± 518.1	<0.005
Active phase	683.2 ± 344.9	280.0 ± 251.7	<0.001
Total oxytocin administered	2708.4 ± 1727.5	1133.7 ± 709.1	<0.001

Abbreviations same as in Table I. Results are mean ± SEM.
*The two-tailed Student *t* test for independent samples.

Table IV. Length of induction and oxytocin requirement in patients with and without fetal breathing movements and low Bishop scores (3 to 5)

	FBM+ (n = 24)	*FBM− (n = 10)*	*p Value**
Induction period (minutes)			
Time to 3 contractions/10 minutes	163.6 ± 92.9	77.0 ± 53.1	<0.01
Latent phase	446.9 ± 268.9	212.5 ± 93.2	<0.02
Active phase	174.8 ± 99.9	79.5 ± 45.5	<0.01
Total induction time	785.2 ± 367.9	369.0 ± 117.0	<0.005
Oxytocin requirement (mIU)			
Oxytocin to 3 contractions/10 minutes	610.3 ± 446.0	234.0 ± 177.7	<0.02
Latent phase	1978.9 ± 386.4	839.0 ± 533.0	<0.03
Active phase	759.2 ± 386.4	345.0 ± 283.6	<0.005
Total oxytocin administered	3348.4 ± 1833.8	1418.0 ± 665.0	<0.003

Abbreviations same as in Table I. Results are mean ± SEM.
*The two-tailed Student *t* test for independent samples.

postterm pregnancies, it was reported that ultrasonographic examination with a 3 cm vertical amniotic fluid pocket resulted in the lowest number of perinatal deaths (1.8/1000), followed by use of contraction stress testing (2.5/1000). Ultrasonography with a 1 cm pocket and biweekly nonstress testing were less effective (5/1000 and 6/1000, respectively).[20] In spite of these measures, however, an increased incidence of meconium-stained amniotic fluid, meconium aspiration syndrome, fetal distress, and low Apgar score was noted.[19, 21, 22] Although induction of labor in postterm pregnancies has generally been indicated,[23] according to recent studies such active management may not be beneficial.[24, 25] The major objections are the increased incidence of prolonged labor and cesarean section when induction is attempted under conditions of an

Table V. Length of induction and oxytocin requirement in patients with and without fetal breathing movements and high Bishop scores

	FBM+ *(n = 13)*	*FBM−* *(n = 14)*	*p* *Value**
Induction period (minutes)			
Time to 3 contractions/10 minutes	66.9 ± 40.0	68.9 ± 44.0	NS
Latent phase	188.8 ± 83.5	135.0 ± 70.0	NS
Active phase	140.3 ± 63.5	80.0 ± 36.0	<0.02
Total induction time	396.1 ± 101.1	283.9 ± 131.7	<0.03
Oxytocin requirement (mIU)			
Oxytocin to 3 contractions/10 minutes	236.1 ± 164.9	212.8 ± 157.0	NS
Latent phase	747.7 ± 318.2	484.3 ± 470.0	NS
Active phase	543.0 ± 194.4	233.5 ± 215.4	<0.001
Total oxytocin administered	1526.9 ± 419.1	930.7 ± 690.6	<0.02

Abbreviations as in Table I. Results are mean ± SEM.

*The two-tailed Student *t* test for independent samples.

unfavorable cervix.[24] One prospective study has revealed, in contrast to previous reports,[4, 17] that cervical examination before induction seems to be a poor screening test to determine the outcome of labor.[26] No other prognostic parameters for labor induction have been described.

Fetal breathing movements have been shown to diminish gradually beginning 72 hours before labor. This may reflect a dual response to increased prostaglandin synthesis, which influences both the fetal respiratory centers and myometrial contractility.

In this study, of the 65 patients with postdate pregnancies who underwent ultrasonographic examination before labor induction, 41 (43%) showed sustained fetal breathing movements, which suggested a low probability of incipient spontaneous labor. It is of interest that 68% of the group with fetal breathing movements had low Bishop scores, in contrast with 42% of the group without fetal breathing movements ($p < 0.03$), indicating a probable connection between fetal breathing activity and ripeness of the uterine cervix. The induction period and the oxytocin requirement were significantly lower in the group without fetal breathing movements than in the group with fetal breathing movements, regardless of the Bishop scores.

The optimal conditions for labor induction were a favorable cervix and absence of fetal breathing activity. Patients with high Bishop scores, whether in the group with or without fetal breathing movements, were relatively easily induced (mean time, 6 hours and 40 minutes, and 4 hours and 44 minutes, respectively). In the absence of a favorable cervical score, fetal breathing movements proved to be an important predictive parameter of inducibility. Thus in patients with low Bishop scores and sustained fetal breathing activity, the mean induction period was 13 hours, more than double that in patients with low Bishop scores and an absence of breathing activity (mean, 6 hours and 10 minutes). The amount of oxytocin administered per time required was higher in the group with fetal breathing movements than in the group without fetal breathing movements (4.3 and 3.8 mU/minutes; the total oxytocin requirement was 60% lower in the group without fetal breathing movements.

In conclusion, fetal breathing activity recorded before induction of labor in postdate pregnancies seems to be a reliable indicator of the time and quantity of oxytocin required throughout labor. Further prospective studies are necessary to confirm these results.

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Risks associated with an elevated maternal serum α-fetoprotein level

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Among 58,187 women tested, 1002 had a maternal serum α-fetoprotein measuring ≥2.5 multiples of the median after correction for race, weight, and insulin-dependent diabetes. They were stratified into three groups: group 1, 2.5 to 2.9; group 2, 3.0 to 5.0; group 3, ≥5.0 multiples of the median. The initial risk of a serious abnormality detected at ultrasonography or amniocentesis was 17% (5%, 12%, and 65% in groups 1, 2, and 3, respectively). After correction for twins and dates, this risk became 23% (7%, 18%, and 71% in groups 1, 2, and 3, respectively). Among the women with high maternal serum α-fetoprotein levels, 556 (77%) had normal ultrasonographic and amniocentesis studies, and the risk of adverse pregnancy outcome was 27% (19%, 29%, and 70% in groups 1, 2, and 3, respectively). There was a statistically significant increase in late fetal and perinatal death, prematurity and growth retardation, oligohydramnios, abruptio placentae, preeclampsia, and congenital abnormalities. The overall risk for abnormality or adverse outcome was 24% in group 1, 41% in group 2, and 91% in group 3. (AM J OBSTET GYNECOL 1991;165:581-6.)

Key words: Neural tube defects, maternal serum α-fetoprotein

Maternal serum α-fetoprotein (AFP) screening was initially designed to identify neural tube defects.[1] This test may also detect other serious abnormalities, including fetal death.[2] In addition, women with high maternal serum AFPs and normal ultrasonographic and amniocentesis studies have shown an increased incidence of adverse pregnancy outcome.[3-5] The purpose of this report is to compare the incidence of open neural tube defects, other fetal abnormalities, or death detected at ultrasonography and amniocentesis and also the risk of poor pregnancy outcome in women with slight, moderate, and greatly elevated maternal serum AFPs.

Material and methods

Patients were identified from two sources. Of these patients 8787 with known pregnancy outcome and 1400 who were tested later were part of a pilot maternal serum AFP screening study carried out between 1980

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Table I. Risk of abnormality detected at ultrasonography or amniocentesis after high maternal serum value

AFP level (multiples of median)	*No.*	*After exclusions*	*Abnormalities*				*Risk of abnormality*	
			Neural tube defects	*Other*	*Death*	*Oligohydramnios**	*Before exclusions (%)*	*After exclusions (%)*
2.5-2.9	393 (39.2%)	267 (37.0%)	7† (1.8%)	5‡ (1.3%)	4 (1.0%)	2 (0.5%)	4.6	6.7
3.0-4.9	473 (47.2%)	328 (45.5%)	15§ (3.2%)	12‖ (2.5%)	29 (6.1%)	2 (0.4%)	12.3	17.7
≥5.0	136 (13.6%)	126 (17.5%)	33¶ (24.3%)	17# (12.5%)	33 (24.3%)	6 (4.4%)	65.4	70.6
TOTAL	1002 (1.7%)	721 (1.2%)	55 (5.5%)	34 (3.4%)	66 (6.6%)	10 (1.0%)	16.5	22.9

*Without other abnormality.

†Anencephaly, 2; spina bifida, 4; encephalocele, 1.

‡Renal agenesis, 1; polycystic kidneys, 1; multicystic kidneys, 1; ventral wall defect, 1; chromosomal abnormality, 1.

§Anencephaly, 5; spina bifida, 9; encephalocele, 1.

‖Multiple congenital abnormality, 2; exstrophy bladder, 1; ventral wall defect, 1; chromosomal abnormality, 6; renal agenesis, 1; duodenal atresia, 1.

¶Anencephaly, 28; spina bifida, 3; hydrocephaly, 2.

#Ventral wall defect, 12; ectopia cordis, 1; renal agenesis, 1; urinary tract obstruction, 1; amniotic bands, 1; cardiac abnormality, 1.

Table II. Categories of poor pregnancy outcome

Oligohydramnios
Death >26 wk
Perinatal death
Delivery <37 wk or birth weight <2500 gm, or both
Abruptio placentae
Preeclampsia
Abnormality

and 1982 (2.0% had a single maternal serum AFP value measuring ≥2.5 multiples of the median). Another 48,000 patients were tested between 1986 and 1989 as part of the California Maternal Serum Screening program. After correction for maternal weight, race, and insulin-dependent diabetes, 1002 (1.7%) had a maternal serum AFP value measuring ≥2.5 multiples of the median. Two hundred eighty-one (28%) were excluded because of too advanced or incorrect dates, a twin pregnancy with maternal serum AFP levels <4.5 multiples of the median, refusal to participate in further testing, and a normal repeat maternal serum AFP level; these women had gestations of <18 weeks when seen, and their first maternal serum AFP value measured between 2.5 and 2.9 multiples of the median. Except for this last group, this study is based on a single high maternal serum AFP. This report is based on the 721 (1.2%) that remained elevated after these exclusions. The women studied were a low-risk group for whom amniocentesis was not indicated because of maternal age, previous neural tube defect, or other reason. The control population was a group of 1100 women who had had a normal maternal serum AFP level and whose pregnancy outcome was known. Both study and control groups included approximately equal numbers of women receiving obstetric care through private obstetricians or health maintenance organizations and comprised few clinic patients. In general, these women sought early obstetric care and were therefore probably at lower risk for low birth weight or prematurity.

Maternal serum AFP determinations were done by radioimmunoassay before 1985 and after 1985 by enzyme immunoassay with the Abbott kit. Assays were done in duplicate and intraassay and interassay variations were <5% and 10%, respectively. All women received genetic counseling, and a complete pregnancy and family history was taken. Each patient underwent a detailed ultrasonographic examination initially and usually additional ultrasonographic examinations later if no explanation for the high maternal serum AFP level was found. Gestational dates were corrected if there was a discrepancy of ≥10 days with menstrual date. Amniotic fluid AFP was measured in the same way as AFP in maternal serum, and all samples were tested with acetylcholinesterase gel electrophoresis and chromosome analysis.

Results

One hundred two women who had two elevated maternal serum AFP levels and 900 who had a single elevated maternal serum AFP value were stratified into three groups by degree of elevation (Table I). Initially, 393 (39.2%) had levels between 2.5 and 2.9 multiples of the median (group 1), 473 (47.2%) between 3.0 and

Table III. Risk of adverse outcome after normal ultrasonography and amniocentesis

AFP (multiples of median)	*No.*	*Death >26 wk*	*Oligohydramnios*	*Prematurity/low birth weight*	*Abruptio placentae*	*Preeclampsia*	*Congenital abnormality*	*Risk of abnormal outcome (%)*
2.5-2.9	249	11 (4.4%)*	3 (1.2%)†	26 (10.4%)‡	1 (0.4%)	1 (0.4%)	4 (1.6%)	18.5
3.0-4.9	270	24 (8.9%)‡	4 (1.5%)§	28 (10.4%)‡	3 (1.1%)	5 (1.9%)	13 (4.8%)‡	28.5
≥5.0	37	5 (13.5%)‡	4 (10.8%)‡	9 (24.3%)‡	2 (5.4%)*	4 (10.8%)‡	2 (5.4%)‖	70.3
TOTAL	556	40 (7.2%)‡	11 (2.0%)‡	63 (11.3%)‡	6 (1.1%)	10 (1.8%)	19 (3.4%)*	26.8
Control risk		1.1%	0.2%	2.3%	0.5%	1.2%	1.1%	6.7

Significance determined by χ^2 test.
*$p < 0.001$.
†$p = 0.016$.
‡$p < 0.0001$.
§$p = 0.004$.
‖$p = 0.019$.

4.9 multiples of the median (group 2), and 136 (13.6%) ≥5.0 multiples of the median (group 3). After 28% were excluded, as already described, 267 (37%), 328 (45.5%), and 126 (17.5%) remained in groups 1, 2, and 3, respectively. Four categories of abnormalities were detected by ultrasonography and amniocentesis: neural tube defects, other serious abnormalities, fetal death, and oligohydramnios. This last category included only cases without another explanation for the oligohydramnios. Abnormalities were listed in one category only; for example, death plus a neural tube defect was included only under neural tube defects. Twins were considered as a single fetus so that abnormalities in one or both were counted only once.

The information of most interest to the primary physician or clinic is the risk of a serious abnormality or neural tube defect at the time of referral and before exclusions at the Prenatal Diagnosis Center. The risk for any abnormality, including death, was 16.5 (4.6%, 12.3%, and 65.4% in groups 1, 2, and 3, respectively). Group 2 contained the most cases of spina bifida and group 3 the most cases of anencephaly. There were four twin pregnancies with a neural tube defect—three in which one fetus had anencephaly and one in which one had spina bifida.

Other serious abnormalities are identified in the footnote to Table I. The overall risk for this group was 3.4% (1.3%, 2.5%, and 12.5% in groups 1, 2, and 3, respectively). All cases of renal agenesis, multicystic kidneys, and urinary tract obstruction had marked oligohydramnios. The overall risk for fetal death detected at the initial ultrasonographic examination was 6.6% (1.0% in group 1, 6.1% in group 2, and 24.3% in group 3). In four cases this affected one twin and in two cases both twins. The overall risk for unexplained oligohydramnios detected at initial ultrasonography was 1.0% (0.5%, 0.4%, and 4.4% in groups 1, 2, and 3, respectively). Six of these aborted spontaneously, and the pregnancy was electively terminated in four others.

Of the 721 women with a high maternal serum AFP level, 556 (77%) had normal ultrasonographic or amniocentesis studies and underwent follow-up to determine the incidence of adverse pregnancy outcome. One of the complications listed in Table II was noted in 26.8%. Fetal or perinatal death was defined as death occurring after 26 weeks and within 28 days of birth; oligohydramnios was moderate or severe and not evident at the initial ultrasonographic examination; prematurity or low birth weight was delivery before 37 weeks or weight <2500 gm or both; abruptio placentae was separation of >15% of the placenta; preeclampsia was a blood pressure of 140/90 mm Hg or an increase in the systolic or diastolic pressure of 20 or 10 mm Hg, respectively, on two readings 6 hours apart, plus proteinuria, edema, or both; a significant congenital abnormality was one not identified at the initial ultrasonographic examination but did not include minor defects such as hydrocele or pilonidal dimple.

Results of the follow-up studies are shown in Table III. There was a statistically significant increase in the incidence of fetal and perinatal death, oligohydramnios, and prematurity or low birth weight in groups 1, 2, and 3. The increased risk was only significant in group 3 for both abruptio placentae and preeclampsia and in group 2 for congenital abnormalities. In the last category the lack of statistical significance is probably explained by the small number of cases in group 3. Death occurred in one twin in one case and in both twins in three cases. Prematurity or low birth weight occurred in one twin in one case (which also had a complete placenta previa) and in both twins in two cases. Fetal death occurred in 8 (73%) of the 11 cases

Table IV. Abnormalities detected in neonatal period

AFP level (multiples of median)	*Risk (%)*
≥2.5-2.9	1.6*†
≥3.0-4.9	4.8‡§
≥5.0	5.4†‖
Overall risk	3.4
Control risk	1.1

*One case each of pyloric stenosis, congenital heart defect, Hirshsprung's disease, double inguinal hernia (three cases of ABO incompatibility not included).

†Not significant.

‡One case each of Weiderman-Beckwith syndrome (premature), clubfoot, cleft lip, cleft palate (death), esophageal atresia, urinary obstruction (oligohydramnios), urinary obstruction (low birth weight), congenital nevus syndrome, diaphragmatic hernia; two cases each of inguinal hernia and congenital heart defect.

§Significantly greater than control risk.

‖One case each of congenital heart defect and posturethral valve.

of severe to moderate oligohydramnios detected after a normal initial ultrasonographic examination and premature delivery occurred in the remainder. Two infants are alive and well, and one continues to have severe respiratory problems.

Details of the congenital abnormalities detected after birth are shown in Table IV. It is important to emphasize that each pregnancy outcome is listed only once and usually under the primary condition. For example, when prematurity and an abnormality occurred, the outcome was listed only once, as a congenital abnormality.

The risk of an adverse pregnancy outcome after a normal ultrasonographic examination and amniocentesis was 18.5%, 28.5%, and 70.2% in groups 1, 2, and 3, respectively. This risk was 88% for a small group of women with maternal serum AFP levels measuring ≥10.0 multiples of the median. The overall risk of an adverse outcome after a high maternal serum AFP value increased from 24% in group 1 to 41.2% in group 2 and 91.3% in group 3 (Table V).

Comment

In this series of 58,187 women, 99.7% of whom had a single maternal serum AFP test, 1.7% had a maternal serum AFP level ≥2.5 multiples of the median after correction for maternal weight, race, and insulin-dependent diabetes, and levels remained elevated after exclusions in 721 (1.2%), mostly because of incorrect dates and twins. The first goal of the study was to compare the risks of an abnormality detected by ultrasonography or amniocentesis in women with mild, moderate, and high elevations of maternal serum AFP. The risk of an open neural tube defect increased from 1.8% in women with maternal serum AFP levels between 2.5 and 2.9 to 24.3% at levels ≥5.0 multiples of the median. Other studies report similar experiences although cutoff levels and comparative degrees of elevation vary. Nelson et al.[3] compared the incidence of neural tube defects in women with maternal serum AFP values between 2.5 and 4.5 multiples of the median and ≥5.0 multiples of the median after two elevated maternal serum AFPs. Of these, 2.4% occurred in the first group and 21% in the second group. Three other reports cite incidences of 6.4% at levels ≥2.0 multiples of the median and 19% and 15% at levels ≥2.5 multiples of the median, usually after two maternal serum AFP tests.[6-8]

In our study 6.7% of women with maternal serum AFP values measuring 2.5 to 2.9 and 70.6% with values ≥5.0 multiples of the median were found to have a serious fetal abnormality at ultrasonography or amniocentesis. Nelson et al.[3] reported an incidence of 4.0% for maternal serum AFP levels between 2.5 and 4.5 and 50% at values ≥5.0 multiples of the median. Their results included one case of placental hemangioma and two abdominal pregnancies. The last complication has been noted previously; Cederquist et al.[9] reported elevated maternal serum AFP values in two of five cases of tubal pregnancy. In our series 21% of the serious abnormalities, excluding fetal death and oligohydramnios, affected the urinary tract; these were identified by ultrasonography. Another 21% were associated with chromosome abnormalities, including two cases of trisomy 18, one case of trisomy 13, two partial monosomies, two partial trisomies, and one with an unidentified marker chromosome. These findings underline the importance of fetal chromosomal analysis after an elevated maternal serum AFP value.

The second part of our study was to determine the risk of an adverse pregnancy outcome in spite of a normal ultrasonographic examination and amniocentesis. It is important to note that there was no evidence of oligohydramnios or other problem at the initial ultrasonographic study. Each case was identified only once, usually in the primary category; for example, if a malformation was complicated by prematurity or death, it was included in the malformation category only. Nelson et al.[3] reported pregnancy complications in 16% of women with maternal serum AFP values measuring 2.5 to 4.5 multiples of the median and 33% at values ≥5.0 multiples of the median after normal amniocentesis results. Other series report increased risks, varying between 25% and 47%.[3, 6-8, 10] We found

Table V. Risk of adverse outcome after high maternal serum AFP value*

AFP level (multiples of median)	*Detected at ultrasonography or amniocentesis (%)*	*After normal ultrasonography or amniocentesis (%)*	*Overall risk*
≥2.5-2.9	6.7	18.5	24.0
≥3.0-4.9	17.7	28.5	41.2
≥5.0	70.6	70.3	91.3
TOTAL	22.9	26.8	43.6

*After exclusions.

a statistically significant increased risk for each type of abnormal outcome compared with the control group of women with normal maternal serum AFPs. Burton[11] reported an overall adverse outcome risk of 26% after an unexplained high maternal serum AFP value, 6% for fetal or neonatal death, 15% for low birth weight, and 6.2% for congenital abnormalities. The increased incidence of congenital malformations may help to explain the relationship of poor pregnancy outcome and unexplained high maternal serum AFP results. With the possible exception of the one case of clubfoot in our series (Table IV), the other malformations most likely resulted from a primary developmental defect arising in the first 2 months of gestation. Their association may be coincidental or the result of a common cause affecting either AFP production or its excretion in the fetus or some pathologic process in the placenta that began in the first trimester and permitted greater transfer into maternal serum. The first two are unlikely in view of the normal amniotic fluid AFP level. The third is supported by a report of a higher incidence of chronic villitis and uterine vascular disease in the placentas of women who had a high maternal serum AFP value compared with those who had normal test results.[12] Additional studies of placental abnormality may help to identify women at greater risk for poor pregnancy outcome.

Although there is general agreement concerning the increased risk of adverse pregnancy outcome after an unexplained high maternal serum AFP value, there is no consensus regarding the need or the method for monitoring these pregnancies. Additonal maternal serum AFP tests are unlikely to distinguish between high-risk and low-risk populations. Hamilton et al.[5] compared pregnancy outcomes in women who had one and two high maternal serum AFP values and found no significant difference in the incidence of problems, although these appeared to be more severe after two elevations. Our adverse outcome risks after a single measurement of maternal serum AFP are consistent with others who used two measurements.[3, 6, 7] In most of these a second test was done within 2 weeks of the first one. Maternal serum AFP values in the third trimester generally have not been helpful in predicting complicated pregnancy outcomes.[13, 14] For the present, repeated ultrasonographic examinations are recommended to detect developing growth retardation, and detailed pathologic studies are recommended on all placentas.

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Study of 156 cases of polyhydramnios and congenital malformations in a series of 118,265 consecutive births

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Polyhydramnios associated with congenital anomalies was studied over nine years in 118,265 consecutive pregnancies. The prevalence of this association was 1.32% (156 cases). A case-control study allowed the examination of genetic and environmental factors for the origin of polyhydramnios associated with congenital malformations. Diagnosis of polyhydramnios associated with congenital malformations was performed prenatally in 41% of the cases; 16% of the infants were stillborn. Fifty-five percent of the cases had more than one malformation, 13.4% of them had a chromosomal aberration, and 32% had multiple malformations that do not constitute a syndrome. There was an increase of consanguinity in the parents of our patients. The incidence of polyhydramnios and congenital anomalies in first-degree relatives was 3.8%, and first-degree relatives had more malformations than the controls had (8.3% vs 3.2%). Our study demonstrated the low capacity of a general prenatal screening program because the diagnosis of malformations associated with polyhydramnios was made in only 41% of the cases and only six of 21 chromosomal abnormalities were diagnosed prenatally. We recommend the use of fetal chromosome analysis and careful ultrasonographic examination in every pregnancy complicated by polyhydramnios. (AM J OBSTET GYNECOL 1991;165:586-90.)

Key words: Polyhydramnios, congenital anomalies, congenital malformations, prenatal diagnosis, chromosonal abnormalities

The incidence of polyhydramnios varies between 0.4% and 1.5%.[2] The diagnosis of polyhydramnios, which was usually made clinically, was associated with an increased frequency of complications in both mother and fetus. In 358 clinically diagnosed cases of polyhydramnios Queenan and Gadow[1] found that 24.6% had diabetes mellitus, 11.5% had erythroblastosis fetalis, 8.4% had multiple births, 1.5% had acute polyhydramnios, and 20% had congenital anomalies; 34% of the cases of polyhydramnios were idiopathic. Since that landmark report, the definition of polyhydramnios has shifted from a clinical to an ultrasonographic one. Here we consider only polyhydramnios associated with congenital malformations. Data on 156 cases of polyhydramnios occurring among 118,265 consecutive pregnancies delivered in Alsace in northeastern France from 1979 to 1987 are reported. A case-control study allowed the examination of genetic and environmental factors for the origin of polyhydramnios associated with congenital malformations. Complete ascertainment of pregnancies with polyhydramnios and congenital malformations in a defined geographic area provides an opportunity to study the epidemiologic association.

Material and methods

The malformations in this study came from 118,265 consecutive births of known outcome, including 814

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Table I. Prevalence of polyhydramnios with one fetal malformation and with more than one fetal malformation

	No.	%	*Prevalence per 10,000 births*	*Sex ratio (male/female)*
Polyhydramnios plus one malformation	70	44.9	5.91	1.18
Polyhydramnios with several malformations	86	55.0	7.27	1.15
Chromosomal abnormalities	21	13.4	1.77	1.33
Nonchromosomal recognized syndromes	15	9.6	1.26	1.14
Multiple malformations without recognized syndromes and without chromosomal abnormalities	50	32.0	2.70	1.08

stillborn babies, registered in our registry of congenital malformations, which covers the "Department du Bas-Rhin" in which Strasbourg is located. Our registry is described elsewhere.[3] Briefly, the newborns of 11 hospitals were examined for the period from Jan. 1, 1979, to Dec. 31, 1987. No deliveries took place at home in the area under study. All newborn children were registered within the first nine days post partum, as were all fetuses with a minimum age of 20 weeks. New cases diagnosed up to 1 year after birth also were registered.

When a suspected or confirmed case was located, the requested information was checked by the doctor in available records: prenatal consultation records, maternity files, neonatal unit files, autopsy reports, outpatient clinic files, and pediatric and surgery files.

Diagnosis of polyhydramnios was made ultrasonographically. All scans were obtained with a real-time sector scanner with the use of a 3.5 to 5.0 or 7.5 MHz transducer of appropriate focal length. The scans were obtained in a routine fashion between 16 and 27 weeks of gestation. Polyhydramnios was diagnosed when more than one pocket of amniotic fluid >7 cm^4 was noted in a vertical dimension. More than 90% of the pregnant women of our region have antenatal ultrasonography.

For each case more than 50 factors included in the registration forms were studied: parity and previous pregnancies, parental age, residence, education, ethnic origin, length, head circumference and weight at birth, inheritance, cytogenetics, occurrence in twins, environmental factors, seasonality, and pregnancy.

For each case a control was studied. The control was a normal child of the same sex born after the case in the same maternity hospital. For seasonality studies the monthly occurrence of all normal births from 1979 to 1987 was chosen as a control rate. For sex ratio, controls were the normal newborn population.

Statistical analysis was obtained by the Statistical Analysis System[5] procedure software package. Statistical comparison was by χ^2 test with Yates' correction for the characteristic under study in cases and in controls and by Student's t test for comparison of means. When it could not be assumed that variances were equal, an adapted t test was used. Odds ratio values were analyzed by χ^2 test.

Results

During the study period 156 cases of polyhydramnios were detected in a total of 118,265 consecutive births, representing an overall prevalence of 1.319 for polyhydramnios associated with congenital malformations detectable at birth and until 1 year of age (Table I). The numbers of cases by year were 15, 14, 21, 25, 26, 14, 11, 12, and 18 from 1979 to 1987, respectively.

One hundred twenty-one children (77.5%) were live births, 25 (16.0%) were stillbirths, and 10 (6.4%) were terminated pregnancies. Diagnosis of malformation was performed at birth in 68 cases (43.6%), during the first week of life in 16 newborns (10.2%), and during the first year of age in eight children (5.1%). Diagnosis of an anomaly was performed prenatally in 64 of 156 cases (41.0%).

There were 70 (44.9%) cases with only one malformation and 86 (55.0%) cases with more than one malformation. The malformations associated with polyhydramnios are listed in Table II. The expected number of each specific malformation was calculated from the frequency of that specific malformation in infants with multiple malformations without polyhydramnios who had been registered during the period from 1979 to 1987; chromosomal abnormalities were excluded. These other malformations allowed us to separate these cases into recognized syndromes (chromosomal and nonchromosomal) and nonrecognized syndromes (multiple malformations) (Table I).

The sex ratios are shown in Table I. Overall sex ratio was 1.16 (84 males, 72 females).

There were no differences between parents of fetuses with polyhydramnios with malformations and control parents with respect to the distribution of parity and previous pregnancies, previous unproductive pregnancies, previous stillbirths, parental age (mean maternal and paternal ages, 27.7 and 30.5, respectively), ethnic origin, residence, and education.

Table II. Malformations in infants born after pregnancy complicated by polyhydramnios, with chromosomal abnormalities excluded and expected malformations by standardization for our registry of congenital anomalies

	No.	*Infants with multiple malformations and polyhydramnios (%)*	*Expected infants with multiple malformations without polyhydramnios (%)*
Congenital heart disease	60	18.7	
Ventricular septal defect	18	5.6	4.6
Atrial septal defect	5	1.5	2.5
Atrioventricular canal	4	1.2	0.07
Ductus arteriosus	5	1.5	1.6
Pulmonary stenosis	2	0.6	—
Aortic stenosis	2	—	—
Mitral stenosis	4	1.2	—
Left heart hypoplasia	3	0.9	—
Dextrocardia	1	—	—
Coarctation of aorta	3	—	—
Other	13	—	—
Skeletal malformations	46	14.3	
Congenital hip dislocation	3	0.9	1.0
Clubfoot	13	4.0	1.8*
Polydactyly	3	0.9	1.6
Syndactyly	4	1.2	1.8
Limb reduction defects	10	3.1	2.4
Other	13	4.0	—
Digestive system	45	4.0	
Esophageal atresia	17	5.3	0.3†
Intestinal atresia	15	4.6	0.8†
Anal atresia	1	—	—
Common mesentery	6	1.8	2.4
Other	6	1.8	—
Renal anomalies	23	7.1	
Hydronephrosis	8	2.5	1.3
Vesicoureteral reflux	3	0.9	1.2
Megaureter	5	1.5	1.3
Renal cyst	1	0.3	1.0
Renal agenesis (unilateral)	1	0.3	1.8
Urethral valve	1	—	—
Other	4	—	—
Cleft	18	5.6	
Cleft palate	11	3.4	2.6
Cleft lip or palate	7	2.2	1.6
Central nervous system	23	7.1	
Anencephaly	1	0.3	0.5
Spina bifida	2	0.6	1.8
Agenesis of corpus callosum	2	—	0.4
Other cerebral agenesis	7	2.2	1.6
Hydrocephalus	9	2.8	2.7
Other	2	—	—
Ocular	10	3.1	
Microphthalmia	3	0.9	0.9
Anophthalmia	1	—	—
Cataract	1	—	—
Other	5	3.1	5.3
Ear, face, and neck	25	7.8	
Facial dysmorphia	8	2.5	1.2
Hypertelorism	10	3.1	1.7
Other	7	2.2	—
Diaphragmatic hernia	13	4.0	1.7*
Abdominal wall	3	0.9	2.4
Genital	2	0.6	3.5
Other	53	16.5	—
TOTAL	321		

Numbers do not sum because some patients had malformations in more than one additional system.

*$p = 0.05$.

†$p = 0.001$.

At birth, the mean weight, length, and head circumference of malformed children with polyhydramnios were, respectively, 3003 gm (controls, 3297; $p < 0.05$), 47.1 cm (controls, 49.4; $p < 0.001$), and 34.4 cm (controls, 34.6; not significant) and the weight of the placenta was 520 gm (controls, 596; $p < 0.05$). Analysis after standardization for sex and gestational age (<36, 36 to 38, and >38 weeks) gave the same conclusions.

Factors associated with malformed fetuses with polyhydramnios

Genetic factors

CONSANGUINITY OF PARENTS. Consanguinity of parents was admitted in 5.1% of the cases (controls, 1.1%; $p < 0.001$). Inherited mendelian conditions were excluded.

An inherited mendelian condition was present in nine cases. Six infants had nonchromosomal syndromes.

CYTOGENETICS. Karotypes were obtained in 80 cases (57.5%). Of these, 59 were normal; the others were as follows: six Down syndrome, 13 trisomy 18 syndrome, and two autosomal deletions (one 18q− and one 4p−). Five of the infants with Down syndrome had no visceral malformation, and one had duodenal atresia and ventricular septal defect. Eleven of the 13 children with trisomy 18 had cardiac defect (ventricular or atrial septal defect, patent ductus arteriosus, bicuspid aortic or pulmonary valves), intestinal malrotation, megaureter, and cleft palate. The two infants with autosomal deletions had facial dysmorphia only. Of the 25 cases in which karyotype was obtained prenatally, only six abnormalities were present (one Down syndrome and five trisomy 18 syndrome).

Among our 156 index patients, four twins (2.5%) were observed (controls, 2.6%). Each twin had an unaffected twin sibling.

One mother and five siblings had the same nonmendelian inherited anomaly as the proband (3.8%), 11 second-degree relatives had the same anomaly as the proband, and 13 first-degree relatives had other malformations (8.3%) (controls, 3.2%, $p < 0.05$).

Environmental factors. Odds ratios for risk factors were evaluated. No significant difference was found between congenital malformations associated with polyhydramnios and controls with regard to the risk factors studied: diabetes, epilepsy, x-ray films, arterial hypertension, fever, "flu," medication, cigarettes, occupational exposure, and parental age (Table III).

Table III. Odds ratio and 95% confidence interval for risk factors compared with controls

Risk factors	*Odds ratio*	*95% Confidence interval*
Diabetes	1.80	0.45-7.15
Epilepsy	1.01	0.99-1.03
X-ray films	0.02	0.006-0.099
Arterial hypertension	0.52	0.21-1.24
Fever	0.029	0.002-0.397
Contraception	0.48	0.18-1.24
"Flu"	0.77	0.28-2.08
Cigarettes	1.15	0.69-1.93
Drugs	0.03	0.005-0.316
Occupational exposure		
Mother	1.61	0.85-3.03
Father	1.35	0.72-2.53
Maternal age	0.63	0.38-1.07
Paternal age	0.67	0.39-1.15

Comment

Polyhydramnios is common, occurring in 0.4% to 1.5% of pregnancies.[1, 2] Congenital anomalies are more common than polyhydramnios.[3] Both are found together in as many as 20% of the cases of polyhydramnios.[4] They should therefore lend themselves to epidemiologic studies. However, there are no unequivocal epidemiologic data on congenital malformations associated with polyhydramnios, because both are difficult to ascertain accurately.

Data on congenital malformations associated with polydramnios have been collected in different ways.[1, 4, 6, 7] In our series >90% of pregnant women had ultrasonographic examination; therefore the overall incidence of congenital malformations associated with polyhydramnios (1.32%) is certainly not too far from the true incidence of this association. The comparison of our data seen in the tables with those of other series shows a lower incidence in the study of Hill et al.[6] (0.98%).

Few series have evaluated an unselected obstetric population for ultrasonographically detectable polyhydramnios.[8, 9] Ten of the 12 cases of Quinlan et al.[9] had a congenital anomaly that led to death in all but two of these infants. Hill et al.[6] detected 102 cases of polyhydramnios among 10,214 patients studied; 10 of these 102 infants were malformed. In the review of pregnancies complicated by hydramnios undertaken by Landy et al.[7] 99 cases were ascertained. Thirteen of them (three being twins or triplets) had associated malformations.

In our series the most common congenital defects associated with polyhydramnios were congenital heart disease, musculo-skeletal malformations, and anomalies of the gastrointestinal tract. This is in agreement with what is known of the physiopathologic features of polyhydramnios.[8, 10, 11] We also noted more diaphragmatic hernias and facial malformations than expected and fewer cases of spina bifida, unilateral renal agenesis, renal cysts, and abdominal wall defects than expected.

Prevalence of chromosomal abnormalities was 1.77% (9.6% of our cases). As this prevalence is not given in the other series it is difficult to know if this prevalence is high or low. In the French nationwide program for prenatal diagnosis of chromosomal abnormalities a new indication was added in 1986, the study of the fetal karyotype after the presence of "signes d'appel" ("calling signs" such as the discovery of a fetal malformation, a small-for-dates fetus, polyhydramnios, or oligohydramnios).[12] Of 2563 fetal karyotypes studied so far, 299 were abnormal (3.7%), which is only about a third of what we have found in our series that took only polyhydramnios into account.

Consanguinity of the parents of fetuses with congenital malformations associated with polyhydramnios is not mentioned in the other studies. In our study 5.1% of the parents were consanguineous. Six first-degree relatives of patients with congenital malformations associated with polyhydramnios had the same nonmendelian inherited malformations. This last frequency is more than twice that of the controls. These conclusions have to be taken into account for genetic counseling.

Maternal epilepsy, hypertension, and fever during pregnancy were no more common among the mothers of the fetuses with congenital malformations associated with polyhydramnios than among controls. During the pregnancy in question 13.1% of the women had threatened abortions, which is significantly different from the controls ($p < 0.01$). Diabetes mellitus was more frequent in the mothers of the children with congenital malformations associated with polyhydramnios than in the controls (4.5% vs 2.1%), but the difference was not statistically significant. In the study of the literature (see Hill et al.[6]) polyhydramnios was associated with maternal diabetes mellitus in 14.7% to 26.3% of cases, in fetuses with or without congenital malformations. In our series polyhydramnios was taken into consideration only when it was associated with fetal malformations. Our study demonstrates that there is no statistically significant increase in diabetes mellitus among the mothers of infants having polyhydramnios associated with congenital malformations.

In our series the diagnosis of congenital malformations was performed prenatally in 41.0% of the cases. In the series of Landy et al.,[7] 99 cases of pregnancy complicated by hydramnios were studied. Prenatal diagnosis of congenital malformations was performed in only seven cases out of 21 with congenital malformations associated with polyhydramnios.

In our patients with chromosomal abnormalities a congenital malformation was detected prenatally at ultrasonographic examination in only one fetus with trisomy 21 syndrome (duodenal atresia) out of six cases and in five with trisomy 18 syndrome out of 13 cases (28.5% of all cases). In the 15 other cases of chromosomal abnormality no anomaly except polyhydramnios was detected at routine ultrasonographic examination. Benacerraf et al.[13] were able to detect 80% of fetuses with trisomy 18 at ultrasonographic examination. In our series seven cases of trisomy 18 could have been detected if level II and III ultrasonographic examinations had been performed, but seven fetuses with chromosomal anomalies (4.5%) (three Down syndrome, two trisomy 18, and two autosomal deletions) would not have had ultrasonographic detection because they had no malformations except mild facial dysmorphia and mental retardation. Therefore we recommend the use of level II or III ultrasonographic examination in every pregnancy complicated by polyhydramnios.[13] When no malformation at level III ultrasonographic examination is detected, fetal chromosome analysis has to be discussed.

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Regulation of atrial natriuretic peptide release in pregnancy: Responses to posture

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The response of plasma atrial natriuretic peptide to three changes in posture that alter atrial stretch was examined in the late phase of pregnancy: (1) after 20 minutes in lateral recumbency, remaining upright for 2 hours decreased median plasma atrial natriuretic peptide values from 37.8 (25th percentile, 22.9 and 75th percentile; 64.2) to 21.8 (10.1 and 28.4) pg/ml in pregnant women ($p < 0.05$) and from 39.2 (27.8 and 51.2) to 24.0 (18.9 and 35.4) pg/ml ($p < 0.001$) in age-matched nonpregnant women; (2) prolongation of lateral recumbency from 20 to 80 minutes increased median plasma atrial natriuretic peptide levels from 31.9 (25.6 and 37.3) to 47.8 (33.0 and 74.6) pg/ml in pregnant women ($p < 0.001$) and from 36.5 (22 and 58.6) to 54.0 (36.3 and 111.7) pg/ml in nonpregnant women ($p < 0.01$); (3) adoption of supine posture during the late phase of normal pregnancy did not alter median plasma atrial natriuretic peptide values significantly [30.4 (26.7 and 42.5) pg/ml during lateral recumbency compared with 34.2 (25.4 and 43.5) pg/ml while supine]. Plasma atrial natriuretic peptide values fell after supine posture in only half the women and supine hypotension occurred in one subject. These studies demonstrate that upright and prolonged lateral recumbent postures significantly influence plasma atrial natriuretic peptide values during pregnancy, as they do in the nonpregnant state, but that supine posture does not alter plasma atrial natriuretic peptide values significantly. Posture must be carefully accounted for in any study of plasma atrial natriuretic peptide in pregnancy. (AM J OBSTET GYNECOL 1991;165:591-5.)

Key words: Atrial natriuretic peptide, pregnancy, posture, supine hypotension

Atrial natriuretic peptide is a peptide hormone synthesized and secreted by atrial cardiocytes. It is natriuretic, diuretic, and opposes the action of many vasoconstrictor substances.[1] In nonpregnant subjects, release of atrial natriuretic peptide is stimulated by atrial stretch,[2] intravascular volume expansion,[3] and oral salt-loading,[4] and is inhibited by upright posture,[5] venesection,[6] ultrafiltration,[7] and salt restriction.[4]

Thus atrial natriuretic peptide has been postulated to have a role in sodium and volume homeostasis and may be important in the regulation of maternal extracellular fluid volume and blood pressure changes during pregnancy. To date, studies of atrial natriuretic peptide in pregnancy have produced conflicting data with some finding basal atrial natriuretic peptide concentrations to be the same as in the nonpregnant state[8, 9] and others disputing this.[10, 11] Moreover, few studies have examined the regulation of this peptide's release during pregnancy, either in response to inhibitory or stimulatory maneuvers.[12-15] Therefore, we sought to examine the regulation of atrial natriuretic peptide in human pregnancy by determining the response of plasma atrial natriuretic peptide to upright, lateral recumbent, and supine recumbent postures, maneuvers known to alter atrial filling.

Methods

Subjects. Third-trimester, normotensive, outpatient, primigravid women were recruited from our antenatal clinic and gave written informed consent. All had singleton pregnancies and were not taking medications other than iron or vitamin supplements. Nonpregnant women were recruited from the nursing and laboratory staff of the same hospital. All subjects were maintained on an unrestricted diet and attended our clinical research room between 10 AM and 2 PM for these studies. The upright posture and lateral recumbent study groups included pregnant and nonpregnant women, whereas the supine recumbent group consisted of pregnant women only. Each subject participated in only one study.

Clinical studies. In each study height and weight were recorded and subjects then voided. After 10 minutes of rest, blood pressure while sitting was recorded in the left arm as systolic and phase IV diastolic blood pressure in pregnant women and phase V diastolic

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Table I. Baseline data

	Ambulation		*Lateral recumbency*		*Supine*
	Pregnant (*n = 10*)	*Nonpregnant* (*n = 21*)	*Pregnant* (*n = 25*)	*Nonpregnant* (*n = 12*)	*Pregnant* (*n = 21*)
Age (yr)	23.9 ± 5.7	26.7 ± 3.0*	25.2 ± 3.2	27.5 ± 3.6	23.8 ± 4.4
Height (cm)	162.4 ± 7.0	164.3 ± 6.5	160.7 ± 8.5	165.6 ± 5.1	157.4 ± 7.7
Weight (kg)	68.8 ± 6.2	58.0 ± 8.7*	70.8 ± 11.4	71.5 ± 16.7	69.4 ± 12.2
Hematocrit	0.33 ± 0.03	0.38 ± 0.02*	0.34 ± 0.03	0.38 ± 0.03*	0.36 ± 0.03
Gestation (wk)	35 ± 3	—	35 ± 2	—	37 ± 1
BP before	107 ± 6	104 ± 8	107 ± 8	118 ± 14†	108 ± 8
	60 ± 7	66 ± 9	68 ± 8	75 ± 9†	70 ± 9
BP after	114 ± 11	107 ± 8	—	—	108 ± 9
	71 ± 9	69 ± 10	—	—	70 ± 5

BP, Blood pressure.

Baseline (prestudy) data and blood pressure before and after changes in posture for pregnant and nonpregnant women in the three posture studies. Data are mean ± SD.

*$p < 0.001$, pregnant vs. nonpregnant.

†$p < 0.05$, pregnant vs. nonpregnant.

blood pressure in nonpregnant women. After 20 minutes in lateral recumbency blood was collected for estimation of hematocrit and plasma atrial natriuretic peptide concentrations.

Upright Posture Study. After 20 minutes in lateral recumbency, subjects remained ambulant for 2 hours and then blood pressure was remeasured and blood was collected for plasma atrial natriuretic peptide evaluation.

Lateral recumbency study. After the initial 20 minutes of lateral recumbency blood was collected as described in this article and then subjects remained in this position for an additional 60 minutes. Blood was collected for remeasurement of plasma atrial natriuretic peptide at this stage.

Supine recumbency study. After 20 minutes in lateral recumbency, pregnant women turned to the supine position for another 20 minutes before the blood pressure and plasma atrial natriuretic peptide were remeasured. In a separate group of five patients, the period of supine posture was prolonged to a total of 2 hours and blood was collected at 30-minute intervals for measurement of plasma atrial natriuretic peptide. Fetal lie and subsequent birth weight were recorded for all participants in this study.

Laboratory measurements. Five milliliters of blood for estimation of plasma atrial natriuretic peptide was collected into a chilled tube that contained ethylenediaminetetraacetic acid (1 mg/ml) and Trasylol aprotinin preparation (50 IU/ml, Bayer) and kept on ice. This was centrifuged at 4°C at 3200 rpm for 15 minutes and the plasma was stored at − 70° C until assay. Plasma atrial natriuretic peptide was determined by radioimmunoassay (Immuno Technology Service Production B.V., The Netherlands) after extraction on C-18 silica columns (Baker) based on the method of Hartter et al.[16] The sensitivity of the assay was 0.8 pg/tube (2.0 pg/ml) and the intraassay and interassay coefficients of variation were 6.7% ($n = 12$) and 9.0% ($n = 6$), respectively. The recovery of synthetic atrial natriuretic peptide added to plasma was 110% for between 50 to 100 pg/ml and 93% for 25 pg/ml. No corrections for recovery were made. Hematocrit value was determined by an automated Coulter counter.

Statistics. Differences between groups were analyzed with the Mann-Whitney test and within groups by the Wilcoxon rank-sum test. Results are expressed as median and 25th and 75th percentiles for atrial natriuretic peptide data and as mean and SD for other data.

Results

Baseline (prestudy) data (Table I). There were no significant differences in the mean age, height, weight, blood pressure, basal plasma atrial natriuretic peptide level, hematocrit value, or stage of gestation among the three groups of pregnant women. Pregnant women in the upright posture study were younger and heavier than their nonpregnant controls and hematocrit value was significantly reduced in pregnant women in both the upright and the lateral recumbency studies.

The median plasma atrial natriuretic peptide concentration for all pregnant subjects (collected after 20 minutes in left lateral recumbency) was 38.6 (25th percentile, 26; 75th percentile, 53.1) pg/ml, which was not significantly different from that of nonpregnant subjects [32.9 (27.9 and 47.8) pg/ml]. Basal plasma atrial natriuretic peptide levels did not differ significantly among the three study groups.

Upright posture study. Plasma atrial natriuretic peptide values decreased significantly from lateral recumbency

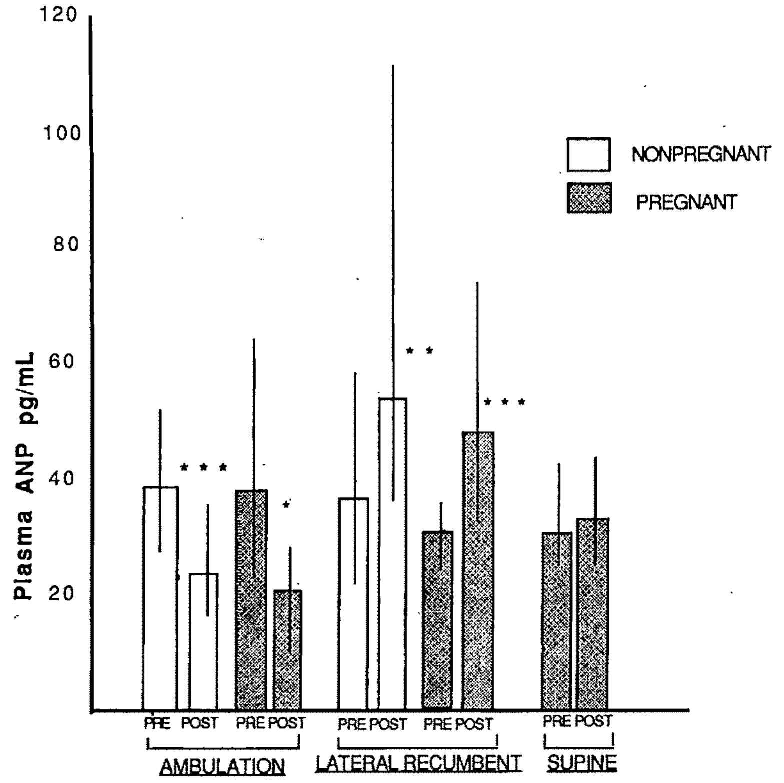

Fig. 1. Plasma atrial natriuretic peptide before and after 2 hours' ambulation, prolonged left lateral recumbency, and supine posture. Data are median and interquartile range. *ANP,* atrial natriuretic peptide. *One asterisk,* $p < 0.05$; *two asterisks,* $p < 0.01$; *three asterisks,* $p < 0.001$ preplasma atrial natriuretic peptide vs postplasma atrial natriuretic peptide.

to upright posture in both the pregnant and the nonpregnant groups (Fig. 1). The median decrease in plasma atrial natriuretic peptide level after 2 hours of upright posture was 11.3 (6.6 and 25.9) pg/ml in pregnant subjects and 10.6 (3.4 and 21.2) pg/ml in nonpregnant subjects.

Systolic blood pressure was not altered significantly by upright posture, although there was a significant increase in diastolic blood pressure in the pregnant group ($p < 0.01$) (Table I).

Lateral recumbency study. Basal plasma atrial natriuretic peptide values after 20 minutes were similar in the pregnant and nonpregnant women (Fig. 1). However, plasma atrial natriuretic peptide levels continued to increase significantly in both groups after a further 60 minutes in lateral recumbency (Fig. 1). The median increase in plasma atrial natriuretic peptide level was similar in pregnant [20.2 (1.0 and 43.8) pg/ml] and nonpregnant subjects [17.5 (8.7 and 44.9) pg/ml].

Supine recumbency study. Basal plasma atrial natriuretic peptide values were not altered significantly from values obtained during lateral recumbency after 20 minutes in supine recumbency in 21 pregnant subjects (Fig. 1). Closer analysis of these plasma atrial natriuretic peptide responses showed that only 12 of these women had a significant decrease in plasma atrial natriuretic peptide level after supine posture [from 35.3 (28.2 and 59.9) pg/ml to 26.2 (20.3 and 35.4) pg/ml ($p < 0.01$)] and the change in plasma atrial natriuretic peptide value was not related to basal plasma atrial natriuretic peptide.

In another five subjects, the period of supine recumbency was prolonged to 2 hours to ensure that this lack of uniform response was not a result of insufficient time spent in supine recumbency. This study demonstrated that the pattern of atrial natriuretic peptide response was always apparent by 30 minutes and that no further changes in plasma atrial natriuretic peptide levels occurred after this time.

Systolic and diastolic blood pressure levels were unaltered by supine posture (Table I) and only one patient experienced an asymptomatic fall in blood pressure from 112/68 to 88/62. Plasma atrial natriuretic peptide level in this patient fell from 28.7 to 16.9 pg/ml. There was a preponderance (70%) of right-lying fetuses, with the fetal head engaged in the pelvis in approximately 50%, and there was only one breech presentation. There was no correlation between fetal lie and plasma atrial natriuretic peptide response to supine posture. The birth weight for infants of women who had a decrease in plasma atrial natriuretic peptide level (3257 gm ± 486 SD) was similar to that of the remaining women (3306 gm ± 254 SD).

Comment

These studies demonstrate that in the late phase of pregnancy, plasma atrial natriuretic peptide value is reduced by upright posture and increased by prolongation of lateral recumbency to 80 minutes, responses that are similar to those of nonpregnant subjects. On the other hand, supine posture reduced plasma atrial natriuretic peptide values only in about half of the pregnant women studied.

It is evident that plasma atrial natriuretic peptide levels varied widely among pregnant women in this study, perhaps reflecting varied sodium intake, a factor known to alter plasma atrial natriuretic peptide values in nonpregnant[4] and pregnant women.[15] However, this study was primarily concerned with evaluation of the direction of change in plasma atrial natriuretic peptide induced by alterations in posture. On the basis of our understanding of atrial natriuretic peptide regulation in nonpregnant subjects, a fall in plasma atrial natriuretic peptide level was expected after upright posture inasmuch as cardiac catheterization studies have demonstrated that right atrial pressure falls by 4 to 7 mm Hg after upright posture.[17] During pregnancy the additional effects of compression of the iliac veins by the gravid uterus might enhance this reduction in atrial pressure during upright posture, although no similar hemodynamic studies have been made. This study confirms that physiologic inhibition of this hormone's release after upright posture is intact during pregnancy although there was no difference in the extent of the fall in plasma atrial natriuretic peptide level between pregnant and nonpregnant women.

Although other researchers have reported a fall in plasma atrial natriuretic peptide values after upright posture in nonpregnant subjects,[5, 18, 19] one study in pregnant women found no significant difference between plasma atrial natriuretic peptide level measured after 15 to 30 minutes seated and that measured after a subsequent 15 to 30 minutes in left lateral recumbency.[15] However, no previous study has examined the effect of prolonged lateral recumbency on plasma atrial natriuretic peptide in normal pregnancy. Our data show that plasma atrial natriuretic peptide level continues to rise significantly when lateral recumbency is extended from 20 to 80 minutes. Others have shown that in nonpregnant sodium-replete subjects, lying recumbent led to an increase in plasma atrial natriuretic peptide that peaked within the first 60 minutes.[20, 21] Others found no significant change in plasma atrial natriuretic peptide level over a 60-minute period of recumbency but this was probably because of the effect of rest before the commencement of the study, which masked the effect of recumbent posture on plasma atrial natriuretic peptide levels.[22] Our study did not determine the exact time for equilibration of plasma atrial natriuretic peptide after lateral recumbency although multiple early blood sampling as well as between 20 and 80 minutes would clarify this issue.

There are three possible explanations for our finding of an increase in plasma atrial natriuretic peptide level with prolonged recumbency. First, there may have been a gradual increase in atrial stretch as venous return increased from 20 to 80 minutes in recumbency although we did not measure central venous pressure directly in this study. Second, it is known that in nonpregnant subjects there is a lag from the time of atrial stretch until the plasma atrial natriuretic peptide level rises. This phenomenon has occurred both after intravenous volume expansion,[3] in which there was a 30-minute lag between peak central venous pressure and peak plasma atrial natriuretic peptide, and after head-down tilt where a 15-minute lag was demonstrated.[22] The explanation for this lag remains speculative but implies the involvement of an intermediate mediator of atrial natriuretic peptide release after atrial stretch.[23] A third explanation may be that prolonged recumbency reduces atrial natriuretic peptide clearance by the kidneys or liver. However, Gillies et al. have shown in males that recumbency usually increases the metabolic clearance rate of atrial natriuretic peptide when compared with sitting or standing[24] and this explanation therefore seems unlikely although there are no studies of the metabolic clearance rate of atrial natriuretic peptide during normal pregnancy.

To determine the relative importance of these three factors during pregnancy it would be necessary to perform simultaneous measurements of central venous pressure and plasma atrial natriuretic peptide during prolonged recumbency. However, on the balance of the available data for nonpregnant subjects, it seems most likely that the increase in plasma atrial natriuretic peptide value after prolonged recumbency in our study reflects a lag between the stimulus of atrial stretch and the peak in plasma atrial natriuretic peptide level.

Finally, supine posture led to a decrease in plasma atrial natriuretic peptide level in only half our third-trimester subjects, implying that a decrease in atrial stretch was perceived only in these subjects. The failure of atrial natriuretic peptide to fall in the remaining subjects was not a result of an inadequate period of supine posture, inasmuch as those who responded with a decrease in plasma atrial natriuretic peptide level did so within the first 30 minutes. Kerr et al. have performed manometric and radiologic studies in the late phase of pregnancy and found a high incidence of complete or partial obstruction of the inferior vena cava by the gravid uterus during supine posture.[25] Lees et al. subsequently studied eight women in the third trimester and showed a mean decrease in right atrial pressure of 3.6 mm Hg when the patient turned from lateral to supine recumbency.[26] Thus it might be anticipated that plasma atrial natriuretic peptide values would fall in all subjects after supine posture but this was not observed. Others believe that fetal position is the important determinant of the degree of caval compression that occurs during supine posture,[27] with engagement of the fetal head in the pelvis minimizing this effect. We found no correlation between fetal lie, birth weight, or gestation and the plasma atrial natriuretic peptide response. It seems most likely that the atrial natriuretic peptide response after supine posture represents a balance between increased venous return from the horizontal position and reduced inferior vena cava flow from uterine-fetal pressure. This must vary among pregnant women and it is therefore unlikely that all pregnant women will exhibit a decreased venous return and thus a fall in plasma atrial natriuretic peptide level after supine recumbency.

In summary, these studies have shown that the normal pregnant woman is able to inhibit the release of atrial natriuretic peptide after upright posture in a manner similar to that of nonpregnant women. After prolonged recumbency in the lateral position there is an increase in plasma atrial natriuretic peptide level and this should be accounted for in studies of atrial natriuretic peptide during pregnancy, particularly when normotensive and hypertensive pregnant women are compared. Finally, plasma atrial natriuretic peptide levels fall in only 50% of pregnant women when the supine position is adopted, probably reflecting the fact that not all experience a fall in right atrial pressure with this maneuver. Overall, the ability to regulate plasma atrial natriuretic peptide after changes in posture is intact during the late phase of normal pregnancy.

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Single and multiple administration of a new triphasic oral contraceptive to women: Pharmacokinetics of ethinyl estradiol and free and total testosterone levels in serum

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Ethinyl estradiol is part of almost every combined oral contraceptive, and its pharmacokinetic characteristics have been thoroughly investigated in numerous studies. However, little is known about its pharmacokinetics during long-term administration, as compared with single-dose administration. In this study 10 women received a triphasic formulation that contained ethinyl estradiol together with the progestin gestodene over one treatment cycle. Mean area under the curve values of ethinyl estradiol were significantly higher on the last treatment day, as compared with the corresponding values obtained from the same women after single-dose administration. However, the observed increase in area under the curve was within the range of pharmacokinetic accumulation, to be expected on the basis of dosing interval and terminal half-life. Another point of interest was the effect of the triphasic preparation on testosterone concentrations in serum. Both total and free testosterone levels were suppressed by about 60% as compared with pretreatment values, and there was no correlation with corresponding sex hormone–binding globulin levels in the serum. (AM J OBSTET GYNECOL 1991;165:596-602.)

Key words: Pharmacokinetics, ethinyl estradiol, chronic administration, testosterone

Gestodene is a new synthetic progestin that, in combination with ethinyl estradiol, is part of a monophasic and a triphasic oral contraceptive. The pharmacokinetics of gestodene after single and multiple administration of the monophasic formulation has been investigated in several clinical studies.[1-3] The pharmacokinetics after single and multiple administration of the triphasic formulation has been reported previously.[4] In this communication the pharmacokinetics of ethinyl estradiol is presented. It was the aim of this study to compare basic pharmacokinetic parameters of ethinyl estradiol in individual subjects after single-dose administration with the corresponding parameters obtained during a complete treatment cycle. In particular, it was of interest to see whether there was an accumulation of ethinyl estradiol in the serum during long-term treatment. In addition, effects of the triphasic formulation on total and free testosterone concentrations were investigated. Since testosterone binds to sex hormone–binding globulin with a high affinity, another aim of this study was to assess any effect of changes in sex hormone–binding globulin concentrations during treatment on total and free testosterone levels in the serum. This could be of interest because reduced free testosterone concentrations could be responsible for a reduction of androgen-dependent side effects.

Material and methods

Study design. The study was designed as an open investigation on 10 healthy women, whose demographic data are presented in Table I. The women had normal biphasic cycles and had not used oral contraceptives for at least 2 months before they participated in the study. All women underwent a thorough physical and gynecologic examination, and informed consent was obtained before their participation.

The women received a single, coated tablet of the triphasic formulation, which contained 0.1 mg gestodene together with 0.03 mg ethinyl estradiol, on day 21 of a pretreatment cycle. Blood samples (5 ml) were taken after an overnight fast, before drug administration, and 0.5, 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 48, and 72 hours after drug intake. A standardized breakfast was served 2 hours after treatment.

After a washout period of 7 days, in a second part of the study, the same women received the following triphasic formulation during a whole cycle: days 1 to 6, 0.05 mg gestodene together with 0.03 mg ethinyl estradiol; days 7 to 11, 0.07 mg gestodene together with 0.04 mg ethinyl estradiol; days 12 to 21, 0.1 mg gestodene together with 0.03 mg ethinyl estradiol.

Blood samples (5 ml) were taken after an overnight

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Table I. Demographic data of 10 women who participated in the study

Patient No.	*Weight (kg)*	*Height (cm)*	*Age (yr)*
1	62	163	28
2	53	157	38
3	57	160	31
4	55	165	25
5	69	172	23
6	64	170	26
7	60	179	33
8	59	156	39
9	60	160	38
10	60	174	36
Mean ± SD	59.9 ± 4.5	165.6 ± 7.8	31.7 ± 6.0

fast on days 2 and 7 before and 0.5, 1, 1.5, 2, 4, 6, 8, 12, and 24 hours after drug administration and on day 21 before and 0.5, 1, 1.5, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after drug intake. A standardized breakfast was served 2 hours after treatment on days 2, 7, and 21.

Additional blood samples were obtained on days 5, 10, 12, 14, 16, 18, and 20 in the morning, before drug administration. All blood samples were kept at 4° C until coagulation; the serum was separated and stored at −20° C until analysis.

Analytic determinations

Determination of ethinyl estradiol: Ethinyl estradiol was measured by a specific radioimmunoassay in all serum samples in duplicate, after an extraction of 0.3 ml serum with diethyl ether. The recovery of the extraction was >90%. Ethinyl estradiol-6,7-^3H (specific activity, 2.2 TBq/mmol; New England Nuclear) was used as tracer; radiochemical purity was >98%. The antiserum (Schering) was obtained by immunization of rabbits with the immunogen ethinyl estradiol-6β-carboxymethyloxime-bovine serum albumin; the final dilution was 1:400,000 in the assay. Details on the specificity of the antiserum are described elsewhere.[5] Extracts from serum samples were dissolved in buffer, antiserum and tracer (5000 counts/min) were added, and the mixture was incubated at 4° C for 16 hours. Bound and free steroids were separated by charcoal treatment, analogous with published procedures.[6] The sensitivity of the standard curve was about 3 to 4 pg ethinyl estradiol per tube, and the lower limit of quantitation was 15 pg/ml. Interassay precision ($n = 5$) was determined with three different control samples, containing ethinyl estradiol 50, 100, and 200 pg/ml plasma, respectively. Experimentally measured concentration values were 56.6 ± 6.0, 120.6 ± 9.1, and 217.6 ± 19.4 pg/ml, respectively. The coefficients of variation of interassay precision were 10.6%, 7.5%, and 8.9%, respectively. Deviations of measured from nomimal values were 13.2%, 20.6%, and 8.8%, respectively.

Other parameters. Total and free concentrations of testosterone and sex hormone–binding globulin and transcortin concentrations in the serum were determined radioimmunologically with commerically available assay kits: total and free testosterone, by solid-phase radioimmunoassays (Coat-A-Count, Diagnostic Products Corporation, Los Angeles), sex hormone–binding globulin, by an immunoradiometric assay (SHBG-IRMA-Count, Diagnostic Products Corporation, Los Angeles); and transcortin, by a radioimmunoassay (IRE Medgenix, Fleurus, Belgium).

Two quality control samples, representing low and high concentrations of the analyte, respectively, were included in each assay and measured in duplicate. Deviation of experimentally measured concentrations from nominal values was ≤16% for all analytes and interassay precision was <10% (coefficient of variation) for total and free testosterone determinations and <15% (coefficient of variation) for sex hormone–binding globulin and transcortin. The lower limits of quantitation were 0.1 ng/ml and 0.2 pg/ml for total and free testosterone, respectively. For sex hormone–binding globulin and transcortin the lower limits of quantitation were 0.5 mmol/L and 0.25 μg/ml, respectively.

Pharmacokinetic and statistical evaluation. Peak serum ethinyl estradiol concentrations and their time points were given as determined. The area under the serum versus time curve was calculated according to the trapezoidal rule. Rate constants of the disposition of ethinyl estradiol in serum and half-lives of its disposition were calculated by means of regression analysis of the logarithm of concentration of the terminal phase (range; 4 to 24 hours after administration). The accumulation factor (R) was calculated as follows:

$$R = \frac{1}{1 - e^{\frac{-\ln 2}{t_{1/2}}\tau}}$$

where $t_{1/2}$ = terminal half-life and τ = dosing interval. Target variables of ethinyl estradiol used for statistical evaluation were areas under the serum versus time

Table II. Concentration of ethinyl estradiol in sera of 10 women after single-dose administration of coated tablet containing 0.1 mg gestodene and 0.03 mg ethinyl estradiol

Sampling time	*Concentration of ethinyl estradiol (pg/ml, mean ± SD)*
Day 21	
0 hr	23.6 ± 13.6
0.5 hr	49.1 ± 27.6
1 hr	83.2 ± 33.6
1.5 hr	94.7 ± 30.4
2 hr	90.5 ± 28.5
4 hr	66.2 ± 12.4
6 hr	47.1 ± 11.8
8 hr	36.4 ± 11.8
10 hr	33.5 ± 8.6
12 hr	34.9 ± 22.0
Day 22, 24 hr	29.2 ± 8.7
Day 23, 48 hr	21.7 ± 10.0
Day 24, 72 hr	20.3 ± 8.7

curve at 0 to 4, 0 to 12, and 0 to 24 hours, obtained from the twenty-first day of the pretreatment and treatment cycles, respectively. The null hypothesis is that there is no difference between day 21 of the pretreatment cycle and day 21 of the treatment cycle with respect to the target variables cited. The alternative hypothesis is that there is a difference between day 21 of the two cycles with respect to a given target variable. With a sample size of 10 subjects, a type I error of 0.05, and a test power of $1 - \beta = 0.9$, a two-sided one sample t test would detect a difference of 1.15 σ.

The correlation coefficients between sex hormone–binding globulin and total free testosterone concentrations for each subject, respectively, were calculated.

Results

Pharmacokinetics of ethinyl estradiol after single- and multiple-dose administration. The time courses (mean ± SD) of the ethinyl estradiol levels observed in the sera of the 10 women after a single dose administration of 0.1 mg gestodene together with 0.03 mg ethinyl estradiol are presented in Table II. Maximum concentrations of 99.5 ± 27.5 pg/ml of ethinyl estradiol were observed after 1.9 ± 0.8 hours. Postmaximum ethinyl estradiol concentrations declined with a mean half-life of disposition of 11.2 ± 6.8 hours. The areas under the serum versus time curve at 0 to 4, 0 to 12, and 0 to 24 hours were 298.8 ± 78.6, 633.9 ± 127.1, and 1018.6 ± 246.1 pg · ml^{-1} · hr, respectively (Table III).

The time courses (mean ± SD) of ethinyl estradiol concentrations observed during the treatment cycle on days 2, 7, and 21 are presented in Table IV. Pharmacokinetic parameters of ethinyl estradiol were calculated on days 2, 7, and 21 of the treatment cycle. Concentration-dependent parameters (peak serum concentration, area under the curve) of ethinyl estradiol were somewhat higher on day 7, probably reflecting the higher dose (0.04 mg) administered (Table III).

On day 21, maximum concentrations of 139.7 ± 40.3 pg/ml were observed 1.6 ± 0.3 hours after administration, and the mean terminal half-life of disposition was 10.8 ± 3.0 hours. Values for the area under the serum versus time curves at 0 to 4, 0 to 12, and 0 to 24 hours were 426.6 ± 100.9, 937.6 ± 193.5, and 1407.0 ± 305.5 pg · ml^{-1} · hr, respectively. The corresponding differences with respect to the initial values on day 21 of the pretreatment cycle were 128 ± 100, 303 ± 211, and 388 ± 440 pg · ml^{-1} · hr, respectively. These were all significantly different from zero ($p < 0.05$).

Total and free testosterone, sex hormone–binding globulin, and transcortin. Sex hormone–binding globulin and transcortin concentrations in the serum before single-dose administration on day 21 of the pretreatment cycle were 90.3 ± 36.0 nmol/L and 37.4 ± 5.2 µg/ml, respectively. On day 21 of the treatment cycle sex hormone–binding globulin and transcortin levels were 198.8 ± 52.0 nmol/L and 84.9 ± 11.4 µg/ml, respectively (Table V).

Mean total and free concentrations of testosterone obtained after single-dose administration and throughout the treatment cycle are presented in Table V. The average correlations (r) between sex hormone–binding globulin and total and free testosterone were 0.21 ± 0.21 and 0.35 ± 0.27, respectively, with a sample size of 10 subjects. As compared with day 21 of the pretreatment cycle, both total and free testosterone concentrations decreased markedly during the treatment cycle (Fig. 1).

Comment

Administration of the triphasic formulation containing gestodene and ethinyl estradiol over one treatment cycle resulted in a 220% elevation of sex hormone–binding globulin concentration as compared with the corresponding pretreatment levels in serum. A similar increase was reported previously for another formulation that contained both hormones at a fixed dose ratio.[3] Total and free testosterone concentrations decreased during treatment in an equal manner, and there was no correlation with corresponding sex hormone–binding globulin levels in the serum. On day 21 of the treatment cycle, an approximate 60% decrease of total and free testosterone concentrations as com-

Table III. Pharmacokinetic parameters (mean ± SD) of ethinyl estradiol obtained on day 21 of pretreatment cycle and on days 2, 7, and 21 of treatment cycle

	Ethinyl estradiol (mg)	*C_{max} (pg/ml)*	*t_{max} (hr)*	*AUC*			*$t_{1/2}$ (hr)*
				0-4 hr (pg · ml^{-1} · hr)	*0-12 hr (pg · ml^{-1} · hr)*	*0-24 hr (pg · ml^{-1} · hr)*	
Pretreatment cycle (n = 6)							
Day 21	0.03	99.5 ± 27.5	1.9 ± 0.8	298.8 ± 78.6	633.9 ± 127.1	1018.6 ± 246.1	11.2 ± 6.8
Treatment cycle							
Day 2 (n = 7)	0.03	107.0 ± 25.3	1.4 ± 0.5	314.7 ± 70.7	681.5 ± 179.8	1016.3 ± 319.8	11.9 ± 2.3
Day 7 (n = 8)	0.04	163.2 ± 46.9	1.2 ± 0.4	487.2 ± 119.1	977.2 ± 193.9	1480.6 ± 305.1	12.3 ± 3.1
Day 21 (n = 9)	0.03	139.7 ± 40.3	1.6 ± 0.3	426.6 ± 100.9	937.6 ± 193.5	1407.0 ± 305.5	10.8 ± 3.0

C_{max}, Peak serum concentration; t_{max}, time point of peak serum concentration; *AUC*, area under the serum versus time curve; $t_{1/2}$, half-life.

Table IV. Concentration of ethinyl estradiol (mean ± SD) in sera of 10 women during treatment cycle with triphasic formulation

Sampling time	*Concentration of ethinyl estradiol (pg/ml)*
0.03 mg ethinyl estradiol	
Day 2	
0 hr	22.8 ± 14.0
0.5 hr	67.1 ± 27.4
1 hr	91.2 ± 29.9
1.5 hr	99.7 ± 23.1
2 hr	96.1 ± 24.4
4 hr	59.9 ± 25.4
6 hr	52.1 ± 19.8
8 hr	45.7 ± 13.5
12 hr	32.7 ± 17.2
Day 3	
24 hr	23.1 ± 11.0
0.04 mg ethinyl estradiol	
Day 7	
0 hr	29.1 ± 15.6
0.5 hr	107.6 ± 55.4
1 hr	160.9 ± 48.9
1.5 hr	150.6 ± 38.9
2 hr	142.6 ± 32.9
4 hr	92.1 ± 14.6
6 hr	66.9 ± 12.8
8 hr	58.0 ± 9.4
12 hr	45.1 ± 17.5
Day 8	
24 hr	38.8 ± 8.4
0.03 mg ethinyl estradiol	
Day 21	
0 hr	37.5 ± 10.1
0.5 hr	64.6 ± 22.0
1 hr	122.7 ± 41.5
1.5 hr	136.4 ± 35.8
2 hr	132.5 ± 34.9
4 hr	89.8 ± 21.9
6 hr	67.2 ± 13.0
8 hr	61.0 ± 14.6
12 hr	51.9 ± 12.5
Day 22	
24 hr	26.4 ± 12.6

pared with the corresponding values on day 21 of the pretreatment cycle was observed. The unbound fraction of testosterone did not change during treatment. The decrease in both total and free testosterone concentrations was therefore due to the impact of the triphasic formulation on the hypothalamic-pituitary-gonadal axis and reflects the decreased production rate of testosterone. A similar observation was reported recently[5] from a study with another gestodene-containing oral contraceptive (0.075 mg gestodene and 0.03 mg ethinyl estradiol). The authors also found a marked decrease by 50% to 70% in free testosterone, but only an approximate 20% to 30% suppression of total testosterone levels in serum. The different extent in suppression of free and total testosterone was attributed by the authors to the rise in sex hormone–binding globulin concentrations. Although similarly high sex hormone–binding globulin levels were reached in this study, both total and free testosterone levels were suppressed to the same extent and showed no correlation with the corresponding sex hormone–binding globulin concentrations.

The increase in transcortin serum levels observed in the present study was identical to the one described for the monophasic gestodene-containing formulation.[7, 8] Transcortin concentrations in the serum are a direct measure for the dose of ethinyl estradiol administered and as such are not influenced by the coadministered progestin. Similar increases have been reported for other formulations containing the same dose of ethinyl estradiol.[3, 10]

When the area under the curve values of ethinyl estradiol were compared on day 21 after a single dose and after multiple-dose administration of the triphasic formulation to 10 women, a significant increase was observed during long-term treatment, although with a considerable interindividual variation. Because ethinyl

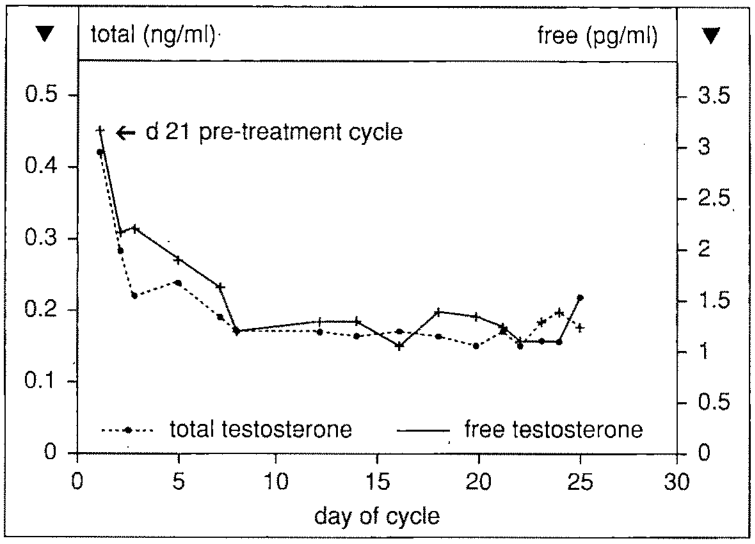

Fig. 1. Mean total and free testosterone concentrations in sera of 10 women who received a triphasic oral contraceptive during one cycle. Samples were obtained before drug administration on respective days.

Table V. Total and free concentration of testosterone and concentrations of sex hormone–binding globulin and transcortin (mean ± SD) in sera of 10 women after single-dose administration and during whole treatment cycle

	Concentration of testosterone			
Day of cycle	*Total (ng/ml)*	*Free (pg/ml)*	*Sex hormone–binding globulin (nmol/L)*	*Transcortin (μg/ml)*
21*	0.42 ± 0.17	3.15 ± 1.62	90.3 ± 36.0	37.4 ± 5.2
2	0.28 ± 0.10	2.10 ± 0.72	109.0 ± 54.3	53.4 ± 9.1
3	0.22 ± 0.11	2.16 ± 0.98	115.8 ± 47.3	70.2 ± 7.3
5	0.24 ± 0.10	1.90 ± 1.15	141.5 ± 43.5	79.5 ± 8.7
7	0.19 ± 0.05	1.61 ± 0.81	163.8 ± 56.9	65.1 ± 9.7
8	0.17 ± 0.09	1.21 ± 0.67	185.0 ± 59.9	64.8 ± 11.8
10	—	—	192.7 ± 42.8	63.8 ± 11.2
12	0.17 ± 0.06	1.29 ± 0.75	197.1 ± 45.6	71.6 ± 16.4
14	0.15 ± 0.08	1.29 ± 0.64	200.6 ± 54.2	72.7 ± 12.3
16	0.17 ± 0.06	1.07 ± 0.47	205.5 ± 58.3	75.1 ± 11.1
18	0.17 ± 0.07	1.36 ± 0.59	193.9 ± 55.5	74.2 ± 13.5
20	0.15 ± 0.07	1.33 ± 0.72	199.2 ± 57.1	80.9 ± 19.1
21	0.17 ± 0.08	1.23 ± 0.51	198.8 ± 52.0	84.9 ± 11.4
22	0.15 ± 0.04	1.09 ± 0.44	212.0 ± 46.3	84.5 ± 13.6
23	0.18 ± 0.09	1.12 ± 0.63	206.2 ± 44.7	84.6 ± 14.7
24	0.20 ± 0.06	1.14 ± 0.54	196.2 ± 41.5	79.4 ± 10.6
25	0.18 ± 0.07	1.53 ± 0.95	209.6 ± 41.6	71.8 ± 8.2

*Single-dose administration.

estradiol is not bound to any significant extent to sex hormone–binding globulin,[11] the concomitant increase in sex hormone–binding globulin concentrations cannot account for this phenomenon. Increasing ethinyl estradiol levels during long-term treatment have already been described during clinical studies with other oral contraceptives.[10, 12, 13] One explanation for the increasing levels was seen in the enterohepatic circulation of this steroid,[12] which during long-term administration might eventually determine steady-state drug levels in the serum. However, the contribution of enterohepatic recirculation of ethinyl estradiol to the total area under the curve was found to be <10%.[14] Another possible reason was thought to be an impairment of hepatic metabolic capacity, caused by an irreversible interaction of ethinyl estradiol with cytochrome P-450 enzymes, which was demonstrated in in vitro experiments.[15] However, so far neither of these explanations has been substantiated. Thus a satisfactory and conclusive explanation for all the observations made in this context has yet to be found.

Another approach is to take into account that the accumulation of ethinyl estradiol in the serum is determined by the ratio of the terminal half-life and the dosing interval. The accumulation can be calculated when both parameters are known. The dosing interval is 24 hours; however, the terminal half-life is a highly variable parameter. A large interindividual variation in terminal half-life (8 to 48 hours) has been reported for ethinyl estradiol,[16] and accordingly one can expect a large variation in ethinyl estradiol-serum levels during long-term treatment. In some individuals who show long half-lives of ethinyl estradiol, an increase in trough levels will be observed, whereas in others with shorter half-lives trough levels will remain practically unchanged. This could account for the interindividual variation in ethinyl estradiol levels during a treatment cycle that was found in this study and also was reported by others.[13]

The half-life determination of ethinyl estradiol is a critical issue. In modern contraceptive formulations it is only administered in low doses of <50 μg/day, the lower limit of determination is usually reached 24 hours after administration. A terminal half-life of disposition longer than about 10 hours therefore cannot be determined with any accuracy, which is a limitation of most published half-life data of ethinyl estradiol, including those presented in this study. In two studies higher doses of ethinyl estradiol were administered,[6, 17] and concentrations in the serum could be measured up to 48 hours after administration. Thus a more reliable evaluation of the terminal half-life of ethinyl estradiol was possible, and mean half-life values of 13.1 and 23 hours, respectively, were reported.

A further complication is the occasional occurrence of a secondary peak in the concentration-time curve observed at about 10 to 12 hours after administration, which is attributed to the enterohepatic circulation of ethinyl estradiol.[14]

In addition to these experimental difficulties in the determination of half-lives of ethinyl estradiol, not only is there the already mentioned large interindividual variation in this parameter but also there is a similarly large intraindividual variation. This has been shown by others[18] and also could be demonstrated in this study. The coefficients of intraindividual variation of the terminal half-life of ethinyl estradiol were in the range of 6% to 40%, and those of interindividual variation were in the range of 19% to 61%.

On the basis of the provisional half-lives of ethinyl estradiol, determined in this study up to 24 hours after a dose, accumulation factors ranging from 1.27 to 1.35 can be calculated, depending on which of the half-life values (10.8 to 12.3 hours) is used. In other words, as compared with the area under the curve values of ethinyl estradiol determined after single-dose administration, an accumulation between 27% and 35% can be expected during long-term treatment. The experimentally determined area under the curve values at the end of long-term treatment were between 38% and 48% higher than corresponding values after a single dose, which is within the theoretical range. However, a correlation between individual terminal half-lives of ethinyl estradiol and corresponding increases in the area under the curve could not be shown, probably because of the above-mentioned limitations of accurate calculation of terminal half-lives after low-dose treatment with ethinyl estradiol. Although other factors, such as enterohepatic circulation or impairment of hepatic metabolic capacity, may possibly contribute to the increase in ethinyl estradiol concentrations observed during long-term treatment, it seems that the observed accumulation of ethinyl estradiol also could be explained by the relation of dosing interval and individual terminal half-life.

In conclusion, this study revealed that there was an increase in ethinyl estradiol concentrations in the sera of women during a treatment cycle with the gestodene-containing triphasic oral contraceptive. Similar observations have been reported previously with other contraceptive formulations containing either gestodene or other progestins in combination with ethinyl estradiol. Although some possible causes for the increase in ethinyl estradiol levels were discussed, further studies are required for a conclusive explanation. During treatment total and free testosterone concentrations in the serum were suppressed to the same extent, although sex hormone–binding globulin levels increased markedly during the treatment cycle. A differential effect on total and free testosterone concentration was not observed.

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Oral tocolysis with magnesium chloride: A randomized controlled prospective clinical trial

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A prospective randomized clinical trial was conducted to assess the efficacy and safety of enteric-coated magnesium chloride (SLOW MAG) as an oral tocolytic agent. Seventy-five patients between 24 and 34 weeks' gestation who were treated with intravenous magnesium sulfate for a first episode of preterm labor were enrolled. After a 12-hour contraction-free period on intravenous therapy, patients were randomized by sealed envelope to one of three groups: group 1, SLOW MAG (535 mg every 4 hours); group 2, oral ritodrine (20 mg every 4 hours); or group 3, no therapy (control). Patients receiving oral therapy were treated until delivery or completion of 36 weeks' gestation. No difference was found between groups with respect to time gained with the use of oral therapy or number completing 36 weeks' gestation. Therapy with enteric-coated magnesium chloride was associated with significantly fewer side effects (20%) as compared with ritodrine (48%) ($p < 0.01$). Our results suggest that compared with ritodrine, enteric-coated magnesium chloride is as effective in prolonging pregnancy and preventing recurrent preterm labor. However, neither enteric-coated magnesium chloride nor ritodrine appeared to be any more effective in the prevention of preterm delivery than observation alone. (AM J OBSTET GYNECOL 1991;165:603-10.)

Key words: Preterm labor, oral tocolysis, magnesium chloride, ritodrine

Prematurity is the leading cause of perinatal mortality in the United States, and the prevention of preterm delivery remains a major goal in obstetrics. Although a number of drugs are used as tocolytic agents in the United States, the effectiveness of these agents in the prevention of preterm delivery is questionable.[1-3] In particular, the role of oral tocolytic agents after successful intravenous therapy is controversial, inasmuch as their efficacy has not been determined.

Many investigators advocate parenteral magnesium sulfate for initial tocolysis because of its reported efficacy and minimal side effects compared with other tocolytic agents.[4-7] At The University of Miami/Jackson Memorial Medical Center, magnesium sulfate is routinely used for initial tocolysis. The dilemma frequently encountered is what if any oral tocolytic agent should be given once premature labor is arrested with parenteral magnesium sulfate. Typically at our institution, patients received the β-adrenergic receptor agonist ritodrine, which carries the same potential for side effects as its intravenous counterpart.[4-7] In addition, it is expensive, with a typical cost to the patient of $210 per week. Because of poor compliance, we therefore sought another agent that would be better tolerated and less costly until the issue of whether oral tocolytic agents were efficacious was resolved.

Historically, oral magnesium salts have not been used because of their known potential for causing gastrointestinal side effects. An enteric-coated form of magnesium chloride (SLOW MAG, Searle & Co., Chicago) is available for use in the United States as a magnesium supplement. SLOW MAG is well-tolerated in nonpregnant patients without the untoward effects typically found with magnesium salts.[8] Each delayed-release tablet of SLOW MAG provides 64 mg (5.26 meq) of elemental magnesium as 535 mg magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$). SLOW MAG is relatively inexpensive (100 tabs for $8.95) with a speculated cost when taken as a tocolytic agent of $2.50 per week. Interest in magnesium as an oral tocolytic agent has recently evolved; however, few clinical trials have been conducted to assess efficacy. A recent study with oral magnesium gluconate showed a relative lack of side effects.[9] However, its efficacy as a tocolytic agent was suggested but not proved.[9] This is the first prospective controlled randomized clinical trial that uses SLOW MAG to assess its efficacy and safety as an oral tocolytic agent.

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Material and methods

At the University of Miami/Jackson Memorial Medical Center, intravenous magnesium sulfate is the first-line tocolytic agent used for all eligible patients admitted with a diagnosis of preterm labor. Preterm labor is defined as either: (1) an initial pelvic examination of 2 cm dilatation in conjunction with two or more contractions per 10 minutes of at least 30 seconds in duration or (2) a change in cervical examination by the same examiner over a 1-hour period in conjunction with two contractions or more per 10 minutes of at least 30 seconds in duration. Gestational age is determined by last menstrual period, early examination, and ultrasonography when available. Ultrasonography is performed on admission to rule out anomalies, confirm dates, and obtain an estimated fetal weight.

Patients excluded from initial intravenous tocolysis with magnesium sulfate are those with any of the following: (1) cervical dilatation ≥4 cm, (2) ruptured membranes, (3) obstetric hemorrhage, (4) chorioamnionitis, (5) preeclampsia, (6) eclampsia, (7) fetal death, or (8) lethal congenital anomaly. Magnesium sulfate is administered as a 4 gm intravenous bolus over 20 minutes, followed by a 2 gm/hour infusion. Serum magnesium levels are obtained before bolus, and then every 6 hours while on intravenous therapy and are not allowed to exceed a level of 8 ng/dl. Infusion rates are adjusted to achieve cessation of contractions, with the maximum infusion rate used being 4 gm/hour. Once contractions have ceased, patients are maintained on a constant infusion of magnesium sulfate for a 12-hour contraction-free period.

Between July 6, 1988, and June 2, 1989, tocolysis was successful with intravenous magnesium sulfate therapy for a first episode of preterm labor on the basis of the above criteria in 75 patients with singleton pregnancies between 24 and 34 completed weeks' gestation who were enrolled in this prospective clinical trial. This study was approved by the Human Subjects Committee at the University of Miami School of Medicine before its initiation. After a 12-hour contraction-free period with intravenous magnesium sulfate therapy, and after giving informed consent, patients were randomized by sealed envelope to one of three groups. Patients in group 1 received 10 mg of oral ritodrine every 2 hours for 24 hours, with the first dose given 30 minutes before discontinuation of the intravenous magnesium sulfate. After the first 24 hours, the ritodrine was changed to 20 mg every 4 hours. Patients in group 2 received one tablet (535 mg) of SLOW MAG every 4 hours, with the first dose given 30 minutes before discontinuation of intravenous magnesium sulfate. Patients in group 3 (observation only) did not receive oral medication, and their intravenous magnesium sulfate infusion was discontinued approximately 30 minutes after randomization. Regardless of group, all patients were observed on the antepartum ward for 48 hours before discharge and were taught the clinical signs and symptoms for preterm labor. All patients were discharged when stable and followed up weekly in a specially designed preterm birth prevention clinic. At each visit the patients were questioned regarding the presence of uterine contractions, low back pain, pelvic pressure, or discharge and were reeducated on the signs and symptoms of labor. Cervical examinations were done at each visit and the patients were monitored for 1 hour with an ambulatory tocodynamometer. The examining physician was blinded to the results of this monitoring until all patients in the study were delivered of infants. All patients were questioned during their clinic visits regarding potential side effects of oral therapy (chest pain, palpitations, tachycardia, headache, fatigue, nausea, vomiting, diarrhea, nervousness). Pulse rate was monitored in all patients. Those who received oral therapy were treated until completion of 36 weeks' gestation or until delivery. Serum magnesium levels were obtained 24 hours after cessation of intravenous magnesium sulfate therapy in all patients. Serum magnesium levels were also obtained weekly from the women who received SLOW MAG.

Patients with recurrent preterm labor were readmitted to the hospital. If the patient still met the defined criteria for intravenous tocolysis at our institution, then intravenous magnesium sulfate therapy was reinstituted. The choice of continued therapy with an oral tocolytic agent was then left to the discretion of the primary physician in attendance.

Among the three groups, we compared time gained in utero, time to and incidence of recurrent preterm labor, and percentage who were delivered of infants after 36 completed weeks' gestation. Time gained in utero was defined as time attributable to the patient's primary assigned therapy, excluding any further time gained in those who had recurrent preterm labor and who again had successful tocolysis. This was done to exclude the possibility of adding bias to the data. Tolerance of the two oral therapies was compared by assessing the incidence, type, and severity of the associated side effects. An analysis of the demographics of the study population was done. Neonatal outcome variables studied included birth weight, gestational age at delivery, Apgar scores, morbidity, and mortality. Serum magnesium levels were compared among groups. Data were analyzed by one-way analysis of variance, the Student t test, χ^2 test, Fisher's exact test, and linear regression as appropriate. A p value of <0.05 was considered significant.

Results

Twenty-five patients were enrolled in each group between July 6, 1988, and June 2, 1989, all of whom were delivered of infants by July 15, 1989. All patients were

Table I. Maternal characteristics

	Ritodrine (*N* = 25)	*SLOW MAG* (*N* = 25)	*Observation* (*N* = 25)	*p Value*
Age (yr)*	22.28 ± 5.02	21.36 ± 5.79	25.00 ± 5.99	NS
Race				
Black American	14	12	11	NS
White Latin	5	7	11	NS
White American	4	3	1	NS
Haitian	2	3	2	NS
Parity				
0	7	7	5	NS
1-2	8	11	12	NS
≥3	10	7	8	NS
Tobacco use	4	3	7	NS
Drug abuse	3	6	3	NS
Prior history of preterm labor, premature rupture of the membranes.	7/25 (28%)	9/25 (36%)	5/25 (20%)	NS

*Data expressed as mean ± SD.

Table II. Treatment response

	Ritodrine (*N* = 25)	*SLOW MAG* (*N* = 25)	*Observation* (*N* = 25)	*p Value*
Onset estimated gestational age*	31.30 ± 2.34	30.86 ± 1.82	30.52 ± 2.57	NS
Onset cervical dilatation (cm)*	1.80 ± 0.96	1.96 ± 0.91	1.56 ± 0.68	NS
Total days gained (intravenous and orally)*	25.16 ± 19.24	30.23 ± 21.89	37.64 ± 28.42	NS
Days gained (orally)*	24.16 ± 19.31	29.28 ± 22.03	31.56 ± 24.39	NS
Recurrent preterm labor (%)	48% (12/25)	44% (11/25)	56% (14/25)	NS
Days to recur*	12.29 ± 11.54	10.92 ± 9.09	13.20 ± 12.72	NS
>36 wk at delivery (%)	56% (14/25)	56% (14/25)	48% (12/25)	NS

*Data expressed as mean ± SD.

of low socioeconomic status. No significant differences were found in maternal age, parity, or race among the three study groups (Table I). Although not statistically significant, more mothers in the SLOW MAG (36%) and ritodrine (28%) groups gave a history of preterm labor with a previous pregnancy than those in the observation group (20%). There was no difference in admitted history of tobacco or substance abuse among groups.

The groups were similar with respect to mean gestational age and cervical dilatation at the onset of preterm labor tocolysis (Table II). No difference was noted in the mean gestational days gained between groups whether considering total days gained (intravenous therapy plus oral therapy) or days gained with oral therapy alone. There was a trend toward more recurrent preterm labor and delivery before completion of 36 weeks' gestation in the observation group compared with the ritodrine and SLOW MAG patients. For patients with recurrent preterm labor, the mean number of days until the recurrence was similar among groups. As illustrated in Fig. 1, no difference occurred among groups in percentage of patients who achieved a 2-, 7-, or 14-day delay in delivery.

Because of a wide range of days gained in each group, we analyzed the data to identify whether cervical dilatation at initial diagnosis of preterm labor affected days gained. Each group had a similar number of patients dilated >2 cm at diagnosis (Table III). Time gained was not significantly different among the groups at >2 cm dilatation, and there was no difference between groups at <2 cm dilatation. However, within the groups, linear regression analysis revealed a negative association between time gained and cervical dilatation at the onset of therapy in the observation group ($r = -0.51$, $p = 0.01$) but not in the SLOW MAG ($r = -0.41, p = 0.05$) or ritodrine groups ($r = -0.32$, $p = 0.12$). The incidence of recurrent preterm labor was not statistically significantly different among groups, regardless of cervical dilatation. However, within all groups patients dilated >2 cm at diagnosis accounted for a higher proportion of recurrent preterm labor.

Thirty-seven of the 75 patients (49%) had a second episode of preterm labor. Of these, 26 were seen in active labor >4 cm dilated and four had premature rupture of membranes (PROM), laboring and delivering spontaneously within 3 days of admission. Tocolysis was successful a second time with intravenous magnesium sulfate therapy in the remaining seven pa-

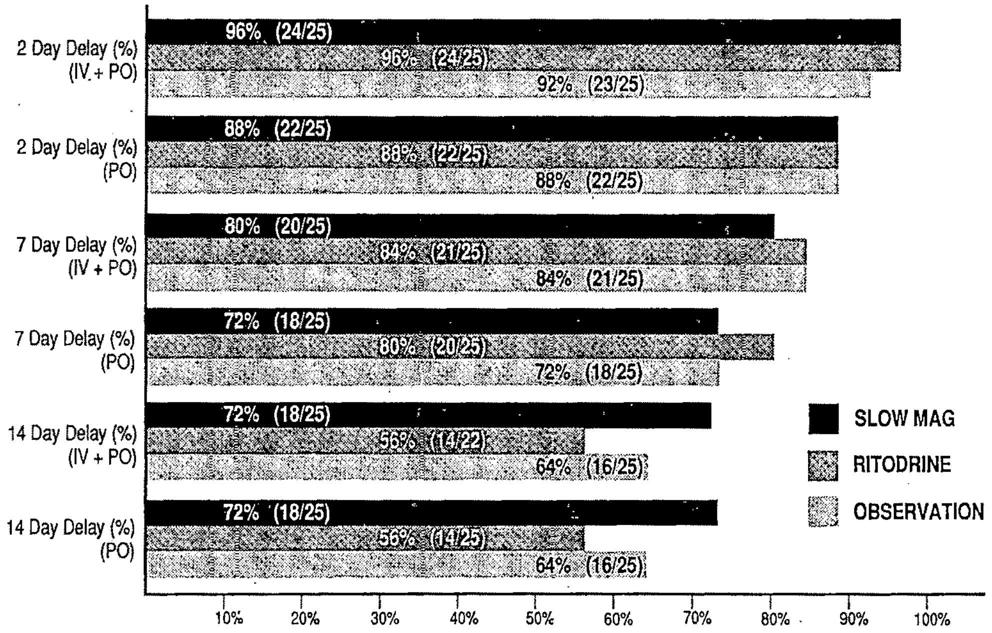

Fig. 1. Comparison of delay in delivery between SLOW MAG, ritodrine, and observation groups.

Table III. Treatment response according to cervical dilatation at onset of therapy for each group

	Days gained (intravenous and orally)*	*Days gained* (orally)*	*Recurrent preterm labor (%)*
<2 cm			
Observation ($N = 13$)	48.97 ± 26.38	40.94 ± 25.10	46 (6/13)
SLOW MAG ($N = 12$)	34.48 ± 23.94	33.74 ± 24.75	33 (4/12)
Ritodrine ($N = 13$)	30.06 ± 22.11	29.17 ± 22.02	42 (5/12)
>2 cm			
Observation ($N = 12$)	25.37 ± 26.20	21.40 ± 19.86	67 (8/12)
SLOW MAG ($N = 13$)	26.30 ± 19.97	24.33 ± 18.70	54 (7/13)
Ritodrine ($N = 12$)	21.26 ± 16.98	20.20 ± 18.66	58 (7/12)

*Data expressed as mean ± SD.

Table IV. Contractions per 1-hour monitoring strip present within 7 days of delivery

	Total (contractions/hr)	*Term (contractions/hr)*	*Preterm (contractions/hr)*
SLOW MAG	6.8 ($N = 14$)	8.4 ($N = 8$)	4.7 ($N = 6$)
Ritodrine	4.4 ($N = 5$)	4.0 ($N = 2$)	4.7 ($N = 3$)
Observation	5.9 ($N = 13$)	7.9 ($N = 7$)	4.5 ($N = 6$)

tients. No patient had more than one episode of recurrent preterm labor.

Sixty-four percent (48/75) of the total study population were monitored for at least 1 hour during their weekly clinic visits. Of these, 16 patients were in the SLOW MAG group, 14 patients were taking ritodrine, and 18 patients were in the observation group. The remaining 27 patients were not monitored, primarily because of delivery before their clinic visit. Technical difficulties as well as noncompliance with clinic visits also made a small contribution. For those monitored, the mean number of strips per patient was 3.48 with a fairly even distribution among groups. Monitoring strips done within 7 days of delivery were available in 32 patients (43%). No difference was found among the three groups in the number of contractions per hour present within 7 days before delivery, whether considering all patients or only those who were delivered of infants before completion of 36 weeks' gestation (Table IV). Although not representative of the whole study population, in the subset of data available monitoring appeared to be of no benefit in predicting which patient was at risk of a recurrence in any of the three groups.

While receiving oral therapy in the hospital and on each weekly clinic visit, the patients who received SLOW MAG and ritodrine were questioned with regard to potential side effects from the medication. Patients who took ritodrine had significantly higher resting pulse rates (96.85 ± 10.73) than did those who took SLOW MAG (82.53 ± 4.73) or no therapy

Table V. Side effects

	*Ritodrine**	*SLOW MAG†*
Nausea	1	2
Vomiting	0	0
Soft stools	0	2
Diarrhea	1	1
Chest pain	0	0
Palpitations, tachycardia	8	0
Headache	0	1
Nervousness	4	0
Fatigue	0	1
Severe enough to stop medication	1/25	2/25

*Total No. of patients with side effects: 12/25 (48%).
†Total No. of patients with side effects: 5/25 (20%).‡
‡$p < 0.05$.

(85.15 ± 4.86) ($p < 0.005$). Table V illustrates the side effects that the patients experienced with their respective medications. Twenty percent (5/25) of patients who took SLOW MAG had side effects, as compared with 48% (12/25) who received ritodrine ($p < 0.01$). Side effects were severe enough to result in discontinuation of ritodrine in one patient because of nervousness and palpitations. In the SLOW MAG group, two patients discontinued the medication, one because of diarrhea and one because of persistant fatigue. It is notable that the latter patient's complaints of fatigue persisted for the duration of her pregnancy.

Serum magnesium levels were compared among groups before intravenous magnesium sulfate, 24 hours after discontinuation of intravenous magnesium sulfate, and weekly in patients taking SLOW MAG (Table VI). There was no significant difference between preintravenous magnesium levels among the three groups. Patients taking SLOW MAG had significantly higher magnesium levels (2.00 ± 0.44) than those of the other two groups. The mean magnesium level for patients taking SLOW MAG from follow-up weekly levels was 1.80 ± 0.16.

The neonatal outcome variables (Table VII) revealed no difference in mean gestational age at delivery or birth weight among groups. The groups were similar with respect to 5-minute Apgar scores, neonatal morbidity and mortality, and hospital stay.

Comment

It is well recognized that intravenous magnesium sulfate is efficacious and safe for initial tocolysis.[4, 5, 10] The purpose of this study was to observe whether orally administered enteric-coated magnesium chloride (SLOW MAG) in patients with arrested preterm labor would prolong gestation and prevent recurrent preterm labor.

In general, studies evaluating the effectiveness of tocolysis in decreasing the rate of preterm birth have shown inconsistent results.[11, 12] In a metaanalysis of 16 controlled clinical trials, initial tocolysis with β-sympathomimetic drugs was found to have a marginal effect in decreasing the preterm delivery rate, and no effect on the perinatal mortality rate.[13] It appears that initial tocolysis provides a short-term delay in delivery, with the best effect in the first 24 hours. One should not expect more than a 48-hour delay in delivery.[14] Of patients who receive only initial therapy, whether with a β-sympathomimetic or magnesium sulfate, 50% will be delivered of infants within 48 hours of discontinuation and no patients should be expected to achieve more than a 7-day delay in delivery.[2]

An oral agent for maintenance therapy has become generally accepted in the United States. Three controlled clinical trials designed to assess the efficacy of continued oral therapy exist in the literature. In the study by Creasy et al.[15] in which preterm labor was arrested with intravenous ritodrine and patients were then randomized to oral ritodrine or placebo, there was no difference in mean gestational age at delivery or in number of days gained after treatment began. An increased number of recurrent preterm labor episodes was, however, found in the placebo group (2.7 vs 1.1), with a fourfold shorter time interval to recurrence than in the ritodrine group, although the exact number of patients who actually had a recurrence in either group is not described. Merkatz et al.[16] reported that patients who received intravenous ritodrine were more likely to achieve a term delivery when continued on oral ritodrine as compared with placebo (52% vs 38%). Patients in whom initial tocolysis was achieved with intravenous alcohol and who then received terbutaline or placebo showed a significant increase in days gained and birth weight in the terbutaline group as reported by Brown and Tejani,[17] but recurrent preterm labor was not discussed specifically. Other investigators have advocated prophylactic oral tocolytic therapy, particularly with β-sympathomimetic drugs, although these studies were not controlled and the effect of the oral therapy per se is difficult to ascertain.[6, 18]

Recently agents other than β-sympathomimetic drugs have been investigated for their oral tocolytic potential. A nonrandomized uncontrolled comparison of magnesium gluconate versus oral ritodrine found equivalent results in the percentage that achieved term delivery (80% for both) and the incidence of recurrent preterm labor (magnesium gluconate 28% vs ritodrine 28%).[19] A criticism of that study was the absence of a control group. In addition, cervical change was not required as part of the definition of preterm labor. Because the success rates reported were higher than those reported by others and ourselves, it is probable that a

Table VI. Serum magnesium levels*

	Ritodrine	*SLOW MAG*	*Observation*	*p Value*
Before intravenous magnesium sulfate	1.60 ± 0.37	1.53 ± 0.31	1.63 ± 0.12	NS
After intravenous magnesium sulfate†	1.65 ± 0.18	2.00 ± 0.44	1.71 ± 0.14	<0.01

*All data expressed as mean ± SD.

†Testing done at least 24 hours after discontinuation of intravenous magnesium sulfate.

Table VII. Neonatal outcome

	Ritodrine	*SLOW MAG*	*Observation*	*p Value*
Delivery estimated gestational age (wk)	34.86 ± 3.72	34.82 ± 3.61	36.00 ± 3.03	NS
Birth weight (gm)	2528 ± 612	2610 ± 759	2766 ± 681	NS
5 min Apgar score	8.52 ± 1.29	8.60 ± 0.722	8.56 ± 0.712	NS
Respiratory distress syndrome	2/25	1/25	0/25	NS
Intraventricular hemorrhage	1/25 (GI)	1/25 (GI)	0/25	NS
Necrotizing enterocolitis	1/25	2/25	0/25	NS
Infection	0/25	0/25	0/25	NS
Death	0/25	2/25§	0/25	NS
Jaundice	10/25	11/25	6/25	NS
Hypermagnesemia	0/25	0/25	0/25	NS
Steroids	2/25	3/25	0/25	NS
Premature rupture of membranes	2/25	4/25	0/25	NS
Hospital stay (day)	7.29 ± 9.06*	6.50 ± 4.89†	6.32 ± 4.89‡	NS

GI, Grade I intraventricular hemorrhage.

*N = 21.

†N = 22.

‡N = 25.

§1 of necrotizing enterocolitis, 1 of respiratory distress syndrome.

large proportion of the patients were never actually in preterm labor.

This study is the first controlled investigation of continued tocolytic therapy with an oral magnesium salt of which we are aware. We found no difference in the ability of SLOW MAG or ritodrine to prolong pregnancy or to achieve delivery after completion of 36 weeks' gestation. Moreover, neither of these two agents was any more effective than close observation alone. In comparison with other studies, we achieved a similar outcome in those who received therapy but a better outcome in our observation group.[15, 16] It has been suggested that oral ritodrine as maintenance therapy is effective in the prevention of recurrent preterm labor.[15, 16] In this study, SLOW MAG proved to be as effective as ritodrine. Our observation group had a longer time to relapse and less recurrences in comparison with the placebo group of prior studies.[15, 16] However, as compared with the ritodrine (48%) and magnesium (44%) groups, there was a trend for more patients in the observation group to have a recurrence of preterm labor (56%). Whether this is a clinically important difference is questionable. Our sample size was not large enough to detect subtle differences in treatment effect. Because the ultimate outcome in all three groups with respect to birth weight, multiple measures of neonatal morbidity, mortality, and hospital stay was the same, it appears this difference had no clinical significance.

A clear difference in time gained in all three groups was observed depending on the patient's cervical examination on admission. Those patients initially dilated >2 cm gained less time. The best success rates with intravenous magnesium sulfate have been reported when cervical dilatation is <2 cm.[4, 5, 10] Those who received intravenous magnesium sulfate and oral ritodrine when initial cervical dilatation is <2 cm can be expected to have a 48-hour delay in delivery 80% of the time, but this falls to 50% if the cervix is dilated >2 cm.[4, 5, 10] From this study, patients who were dilated >2 cm also had a higher incidence of preterm labor recurrence. On linear regression analysis there was an association between the first cervical examination and time gained in the observation group, the greater the dilatation, the less time gained. This association, however, was not seen in the ritodrine or SLOW MAG groups. Perhaps oral therapy is best indicated for those patients whose initial examination shows >2 cm dilation.

It has been suggested that monitoring uterine activity may be beneficial for patients at risk of recurrent preterm labor.[20, 21] In a study of low-risk singleton gestations, on weekly 1-hour uterine tocodynometry, more than six contractions per hour indicated a high risk of preterm labor.[22] Others have found four contractions per hour predictive of impending preterm labor.[23] We attempted to monitor patients weekly for 1 hour to determine whether this would help predict which patients were at higher risk of recurrence. Monitoring strips performed within 7 days of delivery were available in only 43% of the study population. Of those with recurrent preterm labor, strips were available for review of 15 patients evenly distributed across the three groups. The same number of contractions per hour occurred among groups and actually fewer contractions per hour were found than in those patients delivered at term. The benefit of uterine monitoring is controversial. Programs that use it also provide frequent nursing contact and teaching. It may be the combination of patient education and support that contribute to a decrease in preterm birth rather than monitoring alone.[24] In this study, the observation group had a better outcome than that of placebo groups in earlier studies. Perhaps this can be attributed in part to the educational program and close contact that our patients received.

We have shown that SLOW MAG is safe to use in pregnancy and is associated with minimal side effects. Those encountered with oral ritodrine were frequent and confirmed previous reports.[15] No serious consequences from either mode of therapy were encountered. It was necessary to discontinue SLOW MAG in two patients, one because of diarrhea and one because of fatigue. The latter persisted despite discontinuation of the drug. Lethargy as a result of the action of magnesium on the neuromuscular junction and striated muscle is a well-recognized complaint of patients who receive intravenous magnesium sulfate.[6] Given the difference in appearance of ritodrine and SLOW MAG, we believed it would be impossible to have a blinded or placebo-controlled study, therefore bias in the data is possible. Neither of the physicians who actually questioned the patients with regard to side effects (J. M. R. and A. H.) had a vested interest in the SLOW MAG product or knowledge of its potential side effects in the dose prescribed.

SLOW MAG therapy resulted in significant elevation in serum magnesium levels. The mean serum magnesium levels for all patients was on the low side of normal before receiving magnesium sulfate therapy. This has previously been described in patients with preterm labor.[5, 9] It is unknown what serum level of magnesium if any is required for effective oral tocolysis. With intravenous magnesium sulfate therapy, levels are obtained more for safety reasons rather than for therapeutic purposes, inasmuch as no correlation between efficacy and levels has been shown. The dose of SLOW MAG used provided 384 mg of elemental magnesium and mimics that used by Martin.[9, 19]

Given the results of this pilot study one can reasonably conclude that additional controlled clinical trials are needed to justify the continued use of oral tocolytic therapy in the United States. Until this is done, we believe that SLOW MAG offers an alternative to ritodrine for continued oral therapy in patients whose preterm labor has been arrested by intravenous therapy.

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The effects of betamethasone on maternal cellular resistance to infection

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Antenatal administration of glucocorticoids is often used to facilitate fetal lung maturation in cases of prematurity; however, the effects of betamethasone on maternal immune function have not been established. Therefore maternal immune function was assessed with the use of in vitro techniques. Transient and incomplete suppression of the proliferative response to the T-cell mitogen phytohemagglutinin was demonstrated as early as 24 hours after administration of betamethasone. The magnitude and duration of suppression showed a corresponding increase with advancing gestational age, but these effects were not cumulative and were always short-lived (<72 hours). No such suppression of the B-cell mitogen lipopolysaccharide was detected. The nonspecific cellular resistance to infection of maternal monocytes was determined through coincubation with the facultative intracellular pathogen *Listeria monocytogenes*. Increased phagocytic activity with a normal bactericidal effect was measured in the cell preparations obtained from recipients versus nonrecipients of betamethasone. Taken together, these findings clearly show that both specific and nonspecific immune function are intact in the preterm gravid woman after administration of betamethasone and should allay concerns over its use for reasons of infection control alone. (Am J Obstet Gynecol 1991;165:610-5.)

Key words: Fetal lung maturity, preterm labor, corticosteroids, maternal immunity

True preterm labor[1] develops in approximately 7% of gravid women. Of concern is the lack of understanding regarding the cause of this entity, the oftentimes ineffective treatment modalities currently available, and the accompanying complications of preterm delivery that account for some 80% of all neonatal morbidity and mortality.[2]

In an attempt to obviate the respiratory compromise that afflicts the preterm infant, many obstetricians administer betamethasone to the pregnant woman when preterm labor has been arrested. Betamethasone is thought to increase the activity of choline phosphotransferase, thereby enhancing the production of fetal surfactant.[3] With the exception of maternal disorders that contraindicate the safe use of betamethasone (e.g., tuberculosis and peptic ulcer disease), complications of pregnancy per se (e.g., gestational diabetes and pregnancy-associated hypertension) should not limit its use.[4]

The purpose of this study was to further define the appropriateness of betamethasone use during pregnancy by determining its effect on maternal immune function at the cellular level and demonstrating whether the gravid woman is at increased risk for opportunistic infection.

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Material and methods

Patients. The study population was limited to healthy gravid women with a singleton gestation who had first-trimester ultrasonographically confirmed dates between 28 weeks and 32 weeks 6 days of gestation and who met criteria for the diagnosis of preterm labor as described by Creasy and Herron,[5] that is, a minimum cervical dilatation of 2 cm and/or 80% effacement or cervical change accompanied by uterine contractions (four in 20 minutes or eight in 60 minutes). Excluded from the study population were those that had known maternal infection, cervical dilatation ≥ 4 cm at the time of presentation, known congenital anomalies, a lecithin/sphingomyelin ratio ≥ 2.0, or preterm premature rupture of the membranes. Written and verbal informed consent was obtained from all participants in an institution-approved protocol.

Subjects were entered into the study after presentation to the labor and delivery suite when the aforementioned criteria were met. If tocolysis with intravenous magnesium sulfate or subcutaneous terbutaline was successful for at least 2 hours and if delivery, in the judgment of the authors, was unlikely to occur in less than 24 hours, the subject was randomized to be a recipient or nonrecipient of betamethasone. A total of 86 patients were randomized into the study and 50 gestational age–matched patients who were not in labor served as controls. For those designated as recipients, 12 mg of betamethasone was administered intramuscularly and repeated after 12 hours; the drug was then given weekly until 32 completed weeks of gestation.

Peripheral blood processing. Venous blood was drawn from each study subject at the time of randomization and again 24, 48, 72, and 168 hours later. Peripheral blood mononuclear cells were obtained from heparinized (20 U/ml) peripheral blood by sedimentation[6] with Ficoll-Paque (Pharmacia, Piscataway, N.J.) leukocyte separation media. Purified peripheral blood mononuclear cells were washed three times in 50 volumes of complete culture medium by centrifugation at 1200 *g* at room temperature for 10 minutes. The complete culture medium was RPMI 1640 supplemented with 1 mmol/L glutamine, penicillin (100 U/ml), streptomycin (100 µg/ml), 25 mmol/L HEPES, 5×10^{-5} mol/L 2-mercaptoethanol, 12% fetal bovine serum plus 2% horse serum (Reheis Chemical Co., Kankakee, Ill.). Washed peripheral blood mononuclear cells were counted on a hemacytometer, resuspended in complete culture medium at a concentration of 5×10^6/ml, and placed on ice until use.

Monocyte-enriched cell preparations were produced with microexudate-coated tissue culture flasks as described by Ackerman and Douglas.[7]

Lymphoproliferative assay. Cultures were established in triplicate to assess mitogen-induced blastogenesis with peripheral blood mononuclear cells at a density of 1×10^5 per culture in a total volume of 1 ml (No. 3008 multiwell tissue culture plates, Falcon Plastics, Oxnard, Calif.) for 7 days, the last 18 hours of which were in the presence of tritiated thymidine (specific activity, 51 Ci/mmol/L; Research Products International Corp., Elk Grove, Ill.), and stimulated with 10 µg of phytohemagglutinin-P (Difco Laboratories, Detroit) or 25 µg of lipopolysaccharide (*Escherichia coli* 055:B5; Difco). Cultures were harvested onto glass fiber filters, and the incorporation of tritiated thymidine was determined by liquid scintillation spectrophotometry.

Bacteria. *Listeria monocytogenes* strain EGD (Trudeau Institute, Inc., Saranac Lake, N.Y.) was used. A log phase culture (approximately 2×10^8 bacteria per milliliter) was grown in tryptic soy broth and frozen at −20° C in 1 ml aliquots. Before assay the organisms were grown overnight in 5 ml of tryptose phosphate broth, isolated by centrifugation, and diluted in Hanks' balanced salt solution.

Phagocytic assay. The ingestion of *L. monocytogenes* by human monocytes was quantified as described by Czuprynski et al.[8] Briefly, 2.5×10^6 adherent monocytes, 2.5×10^7 bacteria, and 0.1 ml of 50% pooled AB-positive sera in a total volume of 1 ml of Hanks' balanced salt solution admixed in 12 × 75 mm plastic tubes in triplicate. The cultures were rotated in an end-over-end fashion for 30 minutes at 10 rpm at 37° C. After incubation, the cultures were washed three times in Hanks' balanced salt solution by centrifugation at 300 *g* for 10 minutes at 4° C. The cell preparations were centrifuged and treated with Diff-Quick stain (Dade Diagnostics, Aguada, Puerto Rico); intracellular bacteria were enumerated by means of light microscopy. The phagocytic index was calculated as the percentage of monocytes containing at least one bacterium multiplied by the average number of bacteria per positive monocyte.

Bactericidal assay. For measurement of bactericidal activity, admixtures of monocytes (2.5×10^6) and bacteria (2.5×10^6) were incubated as described and rotated for 3 hours at 37° C. Ten volumes of sterile distilled water was added to a sample of the admixture to lyse monocytes and release intracellular bacteria before incubation and again 3 hours later. The samples were plated and incubated, and colonies were counted 48 hours later. The percentage of bacteria killed was determined by dividing the number of bacteria present at 3 hours by the number of bacteria present at time 0.

Statistical analysis. Blastogenesis data were corrected for nonnormality by a square root transformation, and phagocytic and bactericidal data were normalized with an arc sine transformation before statistical evaluation by an analysis of variance and Dunnett's multiple comparison test.[9]

Table I. Effects of betamethasone on maternal lymphoblast transformation

Group	Gestational age* (wk)	No.	*Lymphocyte† (counts/min)* Phytohemagglutinin	*Lymphocyte† (counts/min)* Lipopolysaccharide
1. Control, not in labor	28	10	2,690	15,210
	29	10	2,910	13,990
	30	10	2,940	17,820
	31	10	2,870	17,640
	32	10	2,800	16,930
2. Preterm labor, without betamethasone	28	7	2,970	16,330
	29	8	3,110	15,070
	30	8	3,080	17,090
	31	9	2,950	17,240
	32	10	3,020	16,500
3. Preterm labor, with betamethasone‡	28	8	2,360§	15,820
	29	6	2,250§	16,380
	30	10	1,630§	17,670
	31	11	1,580§	16,440
	32	9	1,560§	17,250

*At time of enrollment.

†Mean of number of samples cultured in triplicate.

‡Recipients of a single course of betamethasone that were undelivered 1 week later. Peripheral blood mononuclear cells were collected and stimulated 24 hours after betamethasone administration.

§Statistically significant at $p < 0.05$, by Dunnett's multiple comparison test.

Table II. Kinetics of suppression of phytohemagglutinin-induced blastogenesis after betamethasone administration

Gestational age* (wk)	No.	*Lymphocytes† after betamethasone (counts/min)* At 0 hr‡	At 24 hr‡	At 48 hr‡	At 72 hr‡	At 168 hr‡
28	8	3020	2200§	2060§	2900	3250
29	6	2840	2100§	2110§	2660	2930
30	10	2990	1580§	1530§	2780	3140
31	11	3110	1670§	1600§	2920	3090
32	9	3060	1620§	1560§	3070	2890

*At time of enrollment.

†Mean of number of samples cultured in triplicate.

‡Recipients of a single course of betamethasone that were undelivered 1 week later. Peripheral blood mononuclear cells were collected and stimulated before administration of betamethasone and 24, 48, 72, and 168 hours after treatment.

§Statistically significant at $p < 0.05$ level by Dunnett's multiple comparison test.

Table III. Cumulative dose-effect of betamethasone on phytohemagglutinin-induced blastogenesis*

Gestational age (wk)	No.	Lymphocytes† (counts/min)
28	8	2220
29	7	2060
30	7	1530‡
31	6	1610‡
32	6	1560‡

*All subjects were enrolled at 28 weeks' gestation, underwent tocolysis, and were treated with betamethasone on a weekly basis. Peripheral blood mononuclear cells were collected and stimulated 24 hours after each dose of betamethasone.

†Mean of number of samples cultured in triplicate.

‡Statistically significant at $p < 0.05$ compared with untreated controls at a similar gestational age but not with gestational age–matched treated subjects, by Dunnett's multiple comparison test.

Results

Mitogen-induced blastogenesis of maternal lymphocytes was used to assess the effect of betamethasone on immune function. As shown in Table I, preterm labor, in and of itself, had no effect on T- or B-cell mitogenesis regardless of gestational age. Lymphocytes from patients with preterm labor treated with betamethasone were normally responsive to stimulation with the B-cell mitogen lipopolysaccharide; however, there was partial suppression of tritiated thymidine incorporation after stimulation with the T-cell mitogen phytohemagglutinin (Table I). Those hyporesponsive lymphocytes were affected as early as 24 hours after maternal treatment with betamethasone, and this effect persisted for at least an additional 24 hours (Table II).

Although the magnitude of suppression did not increase over the 72-hour measurement interval with any

Table IV. Ingestion of *L. monocytogenes* by monocytes from patients in preterm labor

Group	Gestational age* (wk)	No.	Phagocytic index†		
			At 0 hr‡	At 24 hr‡	At 48 hr‡
1. Control, not in labor	28	10	815	ND	ND
	29	10	793	ND	ND
	30	10	754	ND	ND
	31	10	842	ND	ND
	32	10	838	ND	ND
2. Preterm labor, without betamethasone	28	7	762	ND	ND
	29	8	789	ND	ND
	30	8	801	ND	ND
	31	9	759	ND	ND
	32	10	796	ND	ND
3. Preterm labor, with betamethasone	28	8	837	1649§	784
	29	6	773	1388§	829
	30	10	816	1514§	811
	31	11	809	1722§	790
	32	9	766	1709§	790

ND, Not done.

*At time of enrollment.

†Mean of number of samples cultured in triplicate.

‡Recipients of a single course of betamethasone that were undelivered 1 week later. Peripheral blood mononuclear cells were collected and monocyte cultures were established before administration of betamethasone and 24 and 48 hours after treatment.

§Statistically significant at $p < 0.05$ by Dunnett's multiple comparison test.

cell source, lymphocytes obtained from patients at 28 or 29 weeks' gestational age were less affected than those obtained from patients at 30, 31, or 32 weeks (21% to 27% vs 47% to 56%; Table II). All blastogenic responses had normalized by 72 hours and were without further change in magnitude at 168 hours after treatment (Table II). The suppression detected was independent of cell density, length of stimulation, and dose of mitogen (data not shown).

Patients that had successful tocolysis at 28 to 31 weeks' gestation were candidates for repeat doses of betamethasone. The data in Table III indicate that suppression of blastogenic responsiveness was not cumulative, and the kinetics of affect were not altered (data not shown).

Because of the transient depression in T-cell-mediated immunity as determined by the blastogenic response to phytohemagglutinin of lymphocytes obtained from betamethasone recipients, monocytes were obtained from the same subjects and evaluated for resistance to infection with the facultative intracellular bacterium *L. monocytogenes.* The phagocytic index was shown to be enhanced as compared both with control subjects and with nonrecipients of betamethasone at all gestational ages tested (Table IV). These findings were strictly time dependent, peaking at 24 hours after administration of betamethasone with a return to normal levels by 48 hours. In contrast, no difference in bactericidal activity was evident in comparing monocytes derived from gestational age–matched controls and recipients and nonrecipients of betamethasone (Table V). Further, no additional differences in phagocytic indices or bactericidal activities were demonstrated with tests on monocytes obtained from those subjects with successful tocolysis versus those with failure of tocolysis (data not shown).

Comment

The overwhelming consensus of large collaborative trials in the United States and United Kingdom is that antenatal administration of betamethasone to gravid women in preterm labor (≥28 weeks and up to 32 to 34 weeks' gestation) results in a reduction in the incidence of neonatal respiratory distress syndrome.[10-12] Nevertheless, fewer than 50% of obstetricians use betamethasone for the infant at high risk for respiratory distress syndrome.[13] Betamethasone is not universally used for a variety of reasons: (1) its effect is proved only with successful tocolysis, which one cannot easily predict from patient to patient; (2) response to treatment differs with race, gender, and gestational age; (3) it is thought that the potential untoward side effects of the steroid include suppression of maternal immunity with a concomitant increase in maternal and neonatal infection rate.

The maternal immune response clearly changes during the course of pregnancy. Most notably, antibody-mediated immunity remains intact, whereas cell-mediated immunity is altered, presumably through shifts in T-cell subpopulations.[14] However, under normal circumstances it appears that pregnancy does not necessarily render the gravid woman immunocompromised.[15]

Despite the long-held traditional use of steroids for

Table V. Bactericidal activity of monocytes from patients in preterm labor

Group	Gestational age* (wk)	No.	Log_{10} bacteria killed†		
			At 0 hr‡	At 24 hr‡	At 48 hr‡
1. Control, not in labor	28	10	0.49	ND	ND
	29	10	0.52	ND	ND
	30	10	0.53	ND	ND
	31	10	0.56	ND	ND
	32	10	0.48	ND	ND
2. Preterm labor, without betamethasone	28	7	0.57	ND	ND
	29	8	0.51	ND	ND
	30	8	0.55	ND	ND
	31	9	0.46	ND	ND
	32	10	0.55	ND	ND
3. Preterm labor, with betamethasone	28	8	0.60	0.59	0.56
	29	6	0.57	0.49	0.52
	30	10	0.52	0.53	0.51
	31	11	0.53	0.58	0.47
	32	9	0.49	0.55	0.55

ND, Not done.

*At time of enrollment.

†Percentage of bacteria killed during incubation period.

‡Recipients of a single course of betamethasone that were undelivered 1 week later. Peripheral blodd mononuclear cells were collected and monocyte cultures were established before administration of betamethasone and 24 and 48 hours after treatment.

immunosuppressive therapy, the immune system of humans is relatively resistant compared with animal models.[16] The extent and mechanism of action of corticosteroids on the immune response remain to be determined and the relative effects of different dosing regimens are unknown. It is likely that lymphocyte circulation, intrinsic function, and/or elaboration of soluble mediators are all potential targets of action, being affected 4 to 6 hours after a single dose and for up to 24 hours thereafter.[17]

For these reasons we set out to demonstrate what effect, if any, betamethasone administration has on maternal immune function. We confirmed the findings of others[18] that lymphoblast transformation is altered during gestation in a trimester-dependent fashion and that the administration of low doses of corticosteroids further exacerbates this effect on cell-mediated immunity, albeit transiently.

The effects of betamethasone appeared to dominate the T-cell population; therefore we utilized the well-accepted in vitro phagocytic and bactericidal assay of *L. monocytogenes* to evaluate the maternal resistance to infection on a cellular level. We showed increased phagocytic activity with normal bactericidal action in the recipients of betamethasone, which likely represents a compensatory response of monocytes to diminished T-cell activity. This in vitro analogue compares quite well with most other reports in the literature and our own findings, which showed no increase in maternal or neonatal infections in recipients versus nonrecipients of betamethasone (Cunningham DS, Evans EE. Unpublished data), but does differ with at least one study, which showed an increase in maternal infections after betamethasone administration.[19] A prospective comparison of the rates of neonatal sepsis, intraamniotic infection, and postpartum endometritis between recipients and nonrecipients of betamethasone needs to be done.

Recent studies have provided a rational impetus for the adjunctive use of betamethasone with neonatal surfactant[20] and thyrotropin-releasing hormone.[21] Because of this we must continue to document the safety and efficacy of betamethasone for fetal lung maturation. Our data support the safe use of betamethasone in patients in preterm labor with intact membranes, but there remains some concern whether this management scheme is appropriate in the patient in preterm labor with ruptured membranes. Using an approach similar to that in this study one could conceivably put this issue to rest.

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Vasopressin and operative hysteroscopy in the management of delayed postabortion and postpartum bleeding

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Two women, one with refractory postabortion hemorrhage and the other with refractory postpartum hemorrhage, were managed with vasopressin, operative hysteroscopy, and a dilute vasopressin pack. Both patients had been treated by standard methods (i.e., dilation and curettage) and both were being prepared for a surgical intervention procedure. It was decided in both cases to try to slow the bleeding by injection of vasopressin paracervically and then performance of operative hysteroscopy. In both cases, the injection of dilute vasopressin paracervically when coupled with operative hysteroscopy was quite effective in determining the cause of the bleeding and was instrumental in avoiding major operative procedures. (Am J Obstet Gynecol 1991;165:616-8.)

Key words: Vasopressin, operative hysteroscopy

Delayed postabortion and postpartum hemorrhage is uncommon and consequently a problem seldom discussed in obstetrics and gynecology. In fact, there are no articles on extensive review that address the management of refractory delayed postabortion hemorrhage. Few articles have been devoted to delayed postpartum hemorrhage but the management of these patients usually consists of a suction or sharp curettage that when ineffective is followed by intrauterine packing, uterine artery ligation, or even hysterectomy. In some instances, recommendations for angiographic embolization are suggested when suction or sharp curettage is ineffective. Undoubtedly, most physicians would follow a similar approach for delayed postabortion hemorrhage. We have had the opportunity to manage two patients with vasopressin and operative hysteroscopy: one with a delayed refractory postabortion hemorrhage and the other with a delayed postpartum hemorrhage. Both patients were successfully managed and in both instances a major operative procedure that had been planned was avoided. The particulars of each case are presented in the hopes that other patients who are first seen with similar problems might benefit from a similar management scheme.

Case reports

Case 1. The patient was a 27-year-old, gravida 4, para 1, aborta 3 woman who underwent an uneventful outpatient suction curettage for pregnancy termination at 13 weeks' gestation. The procedure was uneventful, and the patient was discharged. Four days after surgery she began to note light bleeding and within a few hours bleeding became copious. She was hospitalized, given three units of packed blood cells, and then taken to surgery where she underwent a sharp curettage of the entire cavity; no tissue was obtained, and the bleeding was only minimally reduced. However, with bed rest as well as methylergonovine maleate, the bleeding subsided over the next 4 hours. She required one additional unit of blood before discharge. After being at home at virtual bed rest for 3 days, she again began to note red vaginal bleeding, which within 24 hours was bright red and profuse. She was taken to the emergency room, transfused with three units of packed blood cells, and then taken to the operating room in anticipation of a laparotomy with either hypogastric artery ligation or hysterectomy. However, before surgery it was decided to try to use the resectoscope along with a paracervical injection of dilute vasopression to determine whether this approach would be of any benefit in determining the cause of bleeding.

After induction of general anesthesia, the patient was placed in position and then prepped and draped in a sterile manner. A weighted speculum was placed in the vagina, and the cervix was firmly grasped with a single-tooth tenaculum. Bright red blood was briskly running out of the cervix. The uterus was firm and judged to be 6 weeks' gestational size. After exposing the vaginal fornices 5 ml of dilute vasopression solution (i.e., 20 units diluted in 30 ml of normal saline solution) was injected paracervically at the 3-o'clock and 9-o'clock positions and within 3 minutes the bleeding dramatically diminished. Then an Olympus 24 French single-channel resectoscope (Olympus Corp., Lake Success, N.Y.) with a wire loop electrode was inserted into the

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uterine cavity. Small clots were cleared with the use of full strength dextran 70 (Hyskon, Pharmacia Inc., Piscataway, N.J.) and the cavity was carefully inspected. A smooth lining was noted without any tissue. However, arterial bleeding was noted along the left uterine wall just above the internal os. The bleeding points were coagulated with a wire loop electrode at a setting of 100 W coagulation. Several additional oozing points that were noted in the fundus were also coagulated. The resectoscope was removed and then approximately 12 inches of 1-inch new gauze packing that had been soaked in the remaining dilute vasopressin solution was firmly packed into the uterus with a long smooth finger forceps. The patient was awakened and transferred to intensive care for observation. Cefazolin (Kefzol; Eli Lilly & Co., Indianapolis, Ind.) 500 mg intravenously every 8 hours and metronidazole (Flagyl; Schiapparelli Searle, Chicago, Ill.) 500 mg intravenously every 6 hours were started. The patient did not bleed through the pack, which was removed 18 hours after surgery. She was continued on bed rest for an additional 2 hours and was then ambulated. She had no further bleeding and was discharged home. Since discharge, which has been approximately 8 months, she has resumed light but normal menses without evidence of any endocrinologic problems.

Case 2. The patient was a 43-year-old, gravida 3, para 3 woman who had undergone a cesarean section at 33 weeks' gestation. She had previous uneventful pregnancies and came in with premature rupture of membranes. She went into labor within 12 hours of admission and because of marked deceleration of the fetal heart rate, a low transverse cesarean section was performed. Closure was in two layers with the use of 0 chromic suture. She was discharged in good condition on the third postoperative day. Twenty days after discharge she began to note intermittent dark red vaginal bleeding. She was treated with bed rest, which proved to be ineffective and within 24 hours the bleeding became bright red and profuse. She was taken to the emergency room where her hemoglobin was noted to be 6 gm. She was tranfused with 3 units of blood and taken to the operating room where a sharp curettage was performed. A moderate amount of tissue was obtained but had no impact on the bleeding. Hysteroscopy was attempted but because of the bleeding was ineffective. A decision was then made to perform a laparotomy with either hypogastric artery ligation or hysterectomy. However, before laparotomy a second decision was made to determine whether a paracervical injection of dilute vasopressin would slow the bleeding to permit reinspection of the uterine cavity with the use of the resectoscope. Within a few minutes after injection of dilute vasopressin (20 units in 30 ml of saline solution), 5 ml at the 3-o'clock and 9-o'clock positions, the bleeding slowed dramatically, and the resectoscope was inserted into the uterine cavity. With the use of sorbitol as the irrigant, several bright red bleeding points were noted on the posterior wall that were coagulated with a rollerball electrode at 100 W coagulation. Large amounts of tissue were present in the anterior fundus. With the use of a combination of a sharp curette and packing forceps, large amounts of placental tissue and amniotic membranes were obtained. After the uterus had been evacuated, the cavity was reinspected and additional bright red bleeding points were noted anteriorly. These were controlled with coagulation. Sixty inches of 1-inch new gauze cotton dressing that had been soaked in a dilute vasopressin pack with a concentration similar to that of the injection was firmly placed in the uterine cavity with a packing forceps. The patient was transferred to intensive care and observed overnight. She had no further bleeding, the pack was removed, and she was ambulated. She had no significant bleeding over the next 24 hours and she was then discharged. Since discharge more than 4 months ago, she has resumed normal menses.

Comment

These two cases show the potential benefit of the resectoscope along with the use of dilute vasoconstrictors and packing in the control of delayed refractory postabortion and postpartum bleeding. With acute postpartum hemorrhage, the use of oxytocin (Wyeth-Ayerst Laboratories, Philadelphia, Pa.) is initially effective if lacerations have been excluded along with retained products of conception. Suction curettage has also been suggested. In patients with delayed postpartum hemorrhage, suction curettage was found to be effective in virtually every case as reported by King,[1] but when ineffective, hysterectomy was used. Alternative to hysterectomy is uterine artery ligation or angiographic embolization.

Packing of the lower uterine segment for control of postcesarean bleeding was reported by Druzin[2] to be effective, although packing has not been a favorite method to control postpartum bleeding. Delayed postabortion bleeding has not been addressed in the literature.

In these two cases, major surgery was contemplated but the interdiction of a dilute vasopressin solution paracervically slowed down the bleeding sufficiently to permit the insertion of a resectoscope, which was instrumental in determining the cause of bleeding and in stopping the bleeding. The benefits of the dilute vasopressin pack remain to be established but it has been found to be effective to control bleeding after resection of submucous myomas, which is the subject of a separate report. It is urged in institutions in which physicians are experienced in the use of the resectoscope that patients who have refractory bleeding after elective abortion and postpartum hemorrhage be managed with a dilute vasopressin injection followed by operative hysteroscopy. Perhaps similar patients can be managed as in these two cases thereby avoiding major operative procedures and even angiographic embolization, which in itself is not totally benign.

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Computerized analysis of episodic changes in fetal heart rate variation in early labor

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Fetal heart rate variation in early labor was measured by computerized analysis in cyclic episodes of low or high variation in 136 women at 37 to 42 weeks' gestation. The amplitude (mean ± SE) in episodes of low variation was 20.6 ± 0.4 milliseconds; in high variation it was 57.3 ± 1.1 milliseconds. The duration (mean ± SE) of low episodes (24.3 ± 1.3 minutes) was less than that of high episodes (45.1 ± 2.7 minutes) but was sometimes >1 hour. In episodes of low variation the amplitude was <5 beats/min long term in 11% and <2.5 milliseconds (pulse intervals) short term in 8%; these measures would be predictive of intrauterine death if persistent before birth. We conclude that the use of decreased fetal heart rate variation as a diagnostic sign of acute fetal hypoxemia in labor is incorrect, because changes of this size occur randomly as a consequence of fetal sleep states. There was no significant correlation between fetal heart rate variation over the last hour of labor and umbilical arterial base deficit on delivery. (AM J OBSTET GYNECOL 1991;165:618-24.)

Key words: Fetal heart rate, fetal behavior, computer analysis, sleep cycles, labor

Many reports show loss of baseline fetal heart rate (FHR) variation to be a reliable sign of impending death before birth.[1,2] From computerized analyses we reached the conclusion that reduced FHR variation was the single most reliable sign of hypoxemia,[3] developing acidemia, and imminent intrauterine death before the onset of labor. There are few direct measurements of FHR variation and its episodic change with fetal states (quiet or active) in early labor near term. It was anticipated that large changes in FHR variation with fetal state, or the presence of prolonged episodes of either state, or diurnal modulation of these phenomena might complicate the interpretation of subsequent changes in FHR pattern with the progress of labor. We therefore undertook a study principally in early labor, ≥2 hours before the onset of the second stage. We chose that phase of labor to avoid the confounding effect of large decelerations that attenuate the measured duration of episodes of low or high FHR variation. We also studied records of labor and delivery that were not complicated by the appearance of large decelerations.

Patients and methods

One hundred thirty-six patients were selected from a group of 500 who required continuous fetal monitoring in labor during the period 1986 through 1988. All patients had a gestational age of >37 weeks. Umbilical arterial blood gas values were available at delivery (measured at 37° C, ABL 3 Radiometer, Copenhagen), and FHR data were available from at least 3 hours before the onset of the second stage of labor. The initial

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FHR analysis was restricted to the early first stage, ≥2 hours before the onset of the second stage. A subset of the data comprised recordings of 51 patients from ≥7 hours before the second stage of labor. On average, recording was begun 7.1 hours before delivery. Table I shows the obstetric characeristics of these patients and the reasons for FHR monitoring. Labor was induced in 95 patients (70%). Within the group 12 patients in whom there were no large decelerations (defined below) were studied throughout the first and second stages of labor.

The maternal age (mean ± SE) was 27.7 ± 0.47 years. No opiate analgesic was administered during the early first stage of labor. The incidence of epidural analgesia was 65% overall, higher in spontaneous labor (83%) than after induction (58%). The duration (mean ± SE) of the first stage was 8.6 ± 0.39 hours, and the second stage was 62.5 ± 5.8 minutes. The birth weight (mean ± SE) was 3.28 ± 0.045 kg (45.7 ± 2.6 percentile[4]), with Apgar scores of 7.5 ± 0.17 at 1 minute and 9.8 ± 0.06 at 5 minutes. At delivery the mean umbilical arterial pH was 7.22 ± 0.008, and the base deficit was 8.6 ± 0.36 mmol/L. Four infants were admitted to the special care baby unit, two because of birth asphyxia and one because of low birth weight. The reason for the fourth baby's admittance is unknown. The final outcome was satisfactory for all. There were no twins.

Table I. Obstetric characteristics of 136 women in labor at 37 to 42 weeks' gestation

	No. of patients	%
Previous events		
Cesarean section	13	10
Stillbirth	5	4
Abortions (>2)	22	16
IUGR	10	7
Current condition		
Hypertension (receiving treatment)	5	4
Proteinuric preeclampsia	12	9
Diabetes (receiving insulin)	4	3
Antepartum hemorrhage	4	3
Other complications	10	7
Smoking >10 cigarettes per day	24	18
Indications for FHR monitoring		
Induction of labor		70
Poor obstetric history		1
Antenatal high risk		4
Suspicious FHR in labor		7
Meconium		4
Bleeding		0
Epidural		32
Intrauterine catheter		1
Synthetic oxytocin (Syntocinon) for augmentation		2
Management of labor		15

IUGR, Intrauterine growth retardation.

FHR analysis. The FHR was recorded (model 8040, Hewlett-Packard Co., Andover, Mass.). The monitors were interfaced to computers (Sage II, Sage Computer Technology, Reno), which sampled the RS 232 port 10 times per second. The FHR signal was recorded by scalp clip electrocardiography in 120 patients (88%), by ultrasonography in one patient, and by both methods, switching to electrocardiography as labor progressed, in the rest. The analysis was not allowed to influence clinical decisions. The pulse interval was validated by an error algorithm[5]; the mean valid intervals were averaged over a period of one sixteenth of a minute (3.75 seconds) and filed with the tocodynamometer measurement. Signal loss was calculated as the proportion of one-sixteenth–minute epochs during which no valid fetal pulse interval measurement was available. The records were of good quality; signal loss (mean ± SE) was 1.8% ± 0.23%.

A baseline was fitted to the FHR record,[6] modified to take account of the more variable basal heart rate encountered in labor, and updated hour by hour. The basal heart rate was measured during episodes of low FHR variation, where present; otherwise, it was calculated from the frequency distribution of pulse intervals to avoid bias through accelerations or decelerations. Accelerations were defined as ≥10 beats/min for 15 seconds' duration. Decelerations were defined as >10 beats/min for >60 seconds' duration or >20 beats/min for >30 seconds' duration. The area of decelerations, below the baseline, was calculated as the number of lost beats.[6]

Long-term FHR variation (band width 16 per minute to 1 per 10 minutes)[7] was calculated as the mean minute range of pulse intervals in milliseconds, excluding decelerations. The mean range of long-term FHR (pulse interval) variation was 45.9 ± 1.1 milliseconds. Episodes of low or high long-term FHR variation were identified as at least 5 of 6 consecutive minutes in which the range of pulse intervals was >32 milliseconds or <30 milliseconds, respectively (e.g., Fig. 1). Short-term FHR variation was calculated as the mean one-sixteenth–minute epoch-epoch variation, excluding decelerations.[7] Analysis was terminated in 6 of the 136 records when large decelerations (>20 lost beats) appeared.

The FHR data were plotted by using the format illustrated previously[6] to check the baseline fit, and also to provide a synoptic display of the variation and numbers of accelerations, decelerations, and uterine contractions every hour for each patient. All results were given as means ± SE unless otherwise stated. Statistical tests were either the Student *t* test or Wilcoxon's matched-pairs signed-rank test, as appropriate.

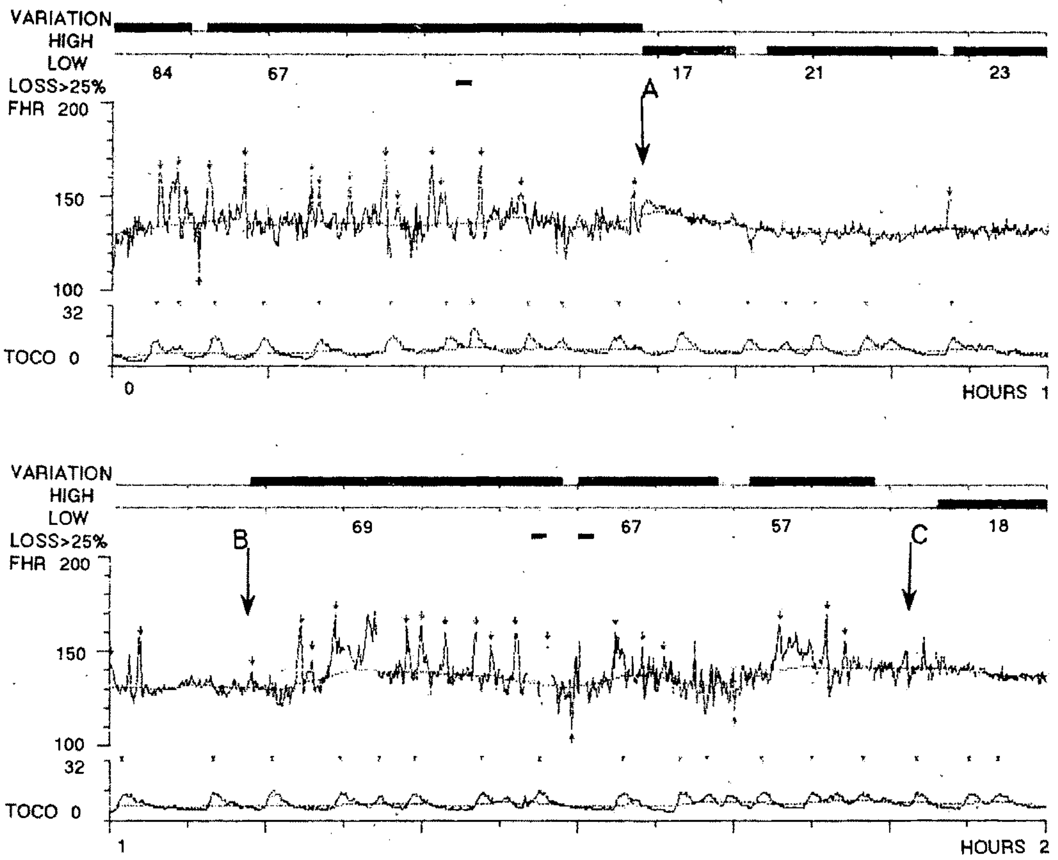

Fig. 1. Computer analysis of 2 hours in the early first stage of labor at 40 weeks' gestation shows successive episodes of low *(A-B)* and high *(B-C)* FHR variation. The mean range of long-term variation was 50 milliseconds, though episodes of low variation averaged 20.7 and high variation averaged 66 milliseconds. No analgesia was given; at delivery, umbilical arterial pH was 7.26 and base deficit was 7 mmol/L.

Results

In 73% of the records there were one or more episodes of both low and high FHR variation. In 23%, there was only one episode of high variation; this was not surprising because these episodes often exceed 1 hour in duration (see below). In 3%, there was only one episode of low variation.

Amplitude of FHR variation in high or low episodes. First, we examined the amplitude of long-term FHR variation in early labor (≥ 2 hours before the onset of the second stage), either spontaneous or induced. To obtain a fair sample, we selected patients whose fetuses were not acidemic at delivery, as judged by the umbilical arterial base deficit (<8 mmol/L), and for whom there were ≥ 2 hours of data available. Table II shows no significant difference in the mean amplitude of FHR variation, in episodes of high or low variation, between 37 patients in whom labor was induced and 18 in whom it occurred spontaneously. For comparison, Table II shows that even in 15 fetuses who had severe metabolic acidemia at delivery (umbilical artery base deficit: range, 13 to 26 mmol/L; mean, 16.4 mmol/L), FHR variation was the same as in the other two groups at this early stage of labor.

In 525 hours of data recorded for all 136 patients, there was no significant correlation between the FHR variation, recorded in episodes of high or low variation, and either gestational age (Fig. 2) or base deficit at delivery. The variation (mean ± SE) in low episodes was 20.6 ± 0.4 milliseconds (range 14.2 to 29.6 milliseconds); in high episodes it was 57.3 ± 1.1 milliseconds. Fig. 2 illustrates the large variation averaged per patient in early labor, from an extreme of 14.2 milliseconds (in low episodes) to 99.4 milliseconds (in high episodes).

In the 91 patient records in which both were available, the ratio (mean ± SE) of long-term FHR variation in episodes of low and high variation was calculated as 0.39 ± 0.008 with a range of 0.23 to 0.73. There was no correlation with base deficit at delivery. The ratio (low/high) was significantly ($p < 0.02$) related to the absolute value of FHR variation in episodes of low variation, but the correlation was low ($r = 0.26$). Hence, there was only a small tendency for those fetuses with

Table II. Comparison of the amplitude of FHR variation in episodes of high or low variation

	No. of patients	*Mean hours per patient*	*Amplitude of long-term FHR variation* (msec)*	
			High variation	*Low variation*
Induced labor (<8 mmol/L base deficit on delivery)	37	4.8	56.0 ± 2.2	20.7 ± 0.6
Spontaneous labor (<8 mmol/L base deficit on delivery)	18	6.1	58.4 ± 3.0	20.5 ± 0.6
Labor ending in severe acidemia (>12 mmol/L base deficit on delivery)	15	3.2	56.7 ± 2.2	21.5 ± 1.0

Blood samples were taken from umbilical artery at delivery.
*None of the mean differences are statistically significant.

lower FHR variation in low episodes to be lower in high episodes.

Measurements of long-term FHR variation (in terms of pulse frequency, beats per minute) in episodes of low FHR variation are normally distributed (Fig. 3). The incidence of measurements of low amplitude (<5 beats/min) was 11.1% of the total (n = 287).

Duration of episodes of high or low FHR variation. The duration (mean ± SE) of episodes of high FHR variation per hour was not significantly different in patients in spontaneous (19.9 ± 1.90 minutes) or induced (21.5 ± 1.14 minutes) labor. Nor was the duration (mean ± SE) of episodes of low FHR variation per hour different in spontaneous (14.5 ± 1.10 minutes) or induced (12.9 ± 0.67 minutes) labor. Because the frequency distributions were not normal, these calculations were repeated with a log transformation, which normalized the distribution and gave the same results, that is, no significant differences. The durations per hour of high or low episodes of FHR variation were not significantly related to outcome as judged by the arterial base deficit at delivery.

We concluded that it was safe to amalgamate the results from all fetuses. Episodes of low FHR variation were selected where bounded at either end by episodes of high variation and vice versa (see Fig. 1, A to B, B to C). Episodes of low variation may be interrupted transiently by a small brief deceleration or acceleration, and episodes of high variation by a brief deceleration; such transient interruptions were ignored for this purpose. There were 172 episodes of high variation in 85 patients, with a duration (mean ± SE) of 45.1 ± 2.7 minutes; there were 123 episodes of low variation in 62 patients, with a duration (mean ± SE) of 24.3 ± 1.3 minutes. Fig. 4 shows the frequency distribution by duration. Episodes of high variation are usually longer, but even episodes of low variation sometimes exceed 60 minutes in early labor. There were four episodes of low variation (3.25%) lasting more than 60 minutes in four different patients (2.9%).

Spencer and Johnson[8] reported the incidence of complete cycles of low and high FHR variation (indicative of quiet or active fetal behavior) to be less common in spontaneous labor (38%) than after induction (68%) at term. Our figures showed an equal incidence in spontaneous (72.5%) and induced (72.7%) labors, judged by the presence of at least one complete cycle of both low and high FHR variation.

Progress of labor and diurnal variation. To find whether the duration or amplitude of FHR variation (in high or low episodes) changed with time in early labor, we selected 51 patients for whom 5 to 12 hours of data were available. Thirty-three of the 51 patients had episodes of high variation, and 18 had episodes of low variation. The linear correlation of each variable (duration and amplitude of high and low variation) with time was calculated for each patient. In 7 of the 33 patients there was a significant ($p < 0.05$ to <0.001) fall in the duration of episodes of high variation with time. This fall was associated with an increase in the incidence of small decelerations. The slope (mean ± SE) (−1.05 min/hr) in the 33 patients overall also was significantly different from zero ($p < 0.05$). The size of the slope was not related to administration of epidural anesthesia or otherwise. None of the other variables (amplitude in episodes of high variation; duration or amplitude in episodes of low variation) were significantly related to time.

The data were also examined in relation to 24-hour clock time. Antenatal observations had shown a large rise in FHR variation in the early evening, from 6:00 PM to midnight, mainly as a result of the increased duration of episodes of high variation.[9] Records for six of the seven patients in whom the duration of episodes of high variation fell significantly in early labor spanned the time period in question. Over this period of time, the mean duration fell in 14 patients (−0.65 min/hr), but not statistically significantly.

Basal FHR and long-term FHR variation. There was an increase in basal FHR in association with episodes of high FHR variation, but the effect was small, a statistically significant ($p < 0.01$) increase of 2.0 ± 0.38

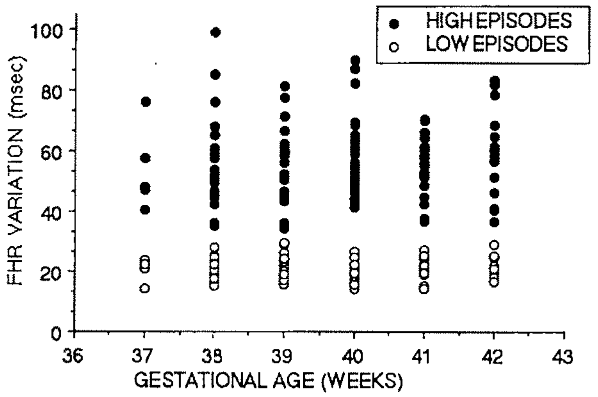

Fig. 2. Distribution of the mean values of long-term FHR variation in episodes of high (●) or low (○) FHR variation with gestational age.

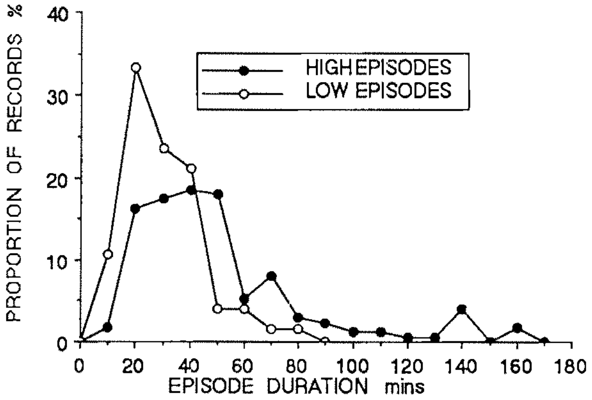

Fig. 4. Frequency distribution of the duration of episodes of high (●) or low (○) FHR variation, evidently skewed.

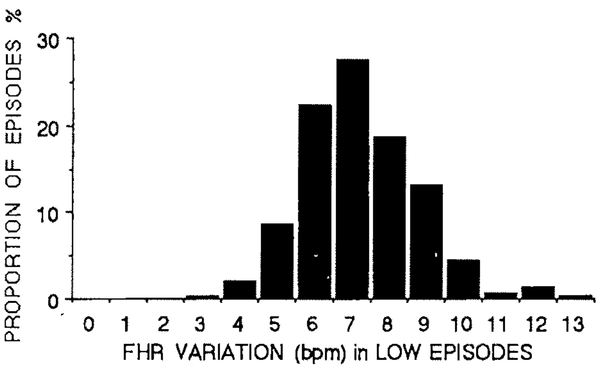

Fig. 3. Frequency distribution of long-term FHR variation (in terms of pulse frequency beats per minute) in episodes of low FHR variation. Eleven percent of these episodes had a mean value of <5 beats/min.

beats/min in episodes of high as compared with low variation, averaged from 51 patients (135 sets of measurements; high-low-high or vice versa). There was no relation between the size of the increase and the basal rate in low episodes.

At the start of recording, the mean basal FHR (mean ± SD) was 138 ± 8.0 beats/min; there was a small downward trend, statistically nonsignificant, with gestational age (37 to 42 weeks). During the course of the early first stage of labor (a period of 2 to 10 hours, before the onset of large decelerations or the last 2 hours before the second stage), FHR rose by 3.7 ± 11 beats/min. However the range of change was large, from −13 to +43 beats/min. The frequency distribution of changes was approximately normal. In 11 fetuses at 39 to 42 weeks' gestation, there was a rise in FHR by 20 to 43 beats/min to values of 154 to 180 beats/min; all were in mothers who had received epidural analgesia. Yet there were continued episodic changes in FHR variation; the arterial base deficit on delivery was relatively low (mean, 7.0 mmol/L, range 3 to 12 mmol/L).

Short- and long-term FHR variation. When long-term FHR variation is attenuated before birth (as in fetuses with intrauterine growth retardation and reduced end-diastolic umbilical blood flow velocity), both short- and long-term FHR variation must be measured.[7] Occasionally, there is a slow, sinusoidal rhythm of amplitude sufficient to constitute high variation superimposed on an otherwise flat trace, not uncommonly before death in utero. Measurement of short-term FHR variation is therefore a better guide to the identification of terminal traces (<2.5 millisecond short-term variation). The ratio (mean ± SD; short term/long term) in early labor was 0.20 ± 0.039, $r = 0.74$, consistent with antenatal observations. Ten of the 51 patients with records of greater length had episodes of short-term low FHR variation ≤2.5 milliseconds; in 5 patients, there were 5 to 7 such episodes lasting 5 to 28 minutes. Their presence was not related to a high base deficit on delivery (mean 7.5 mmol/L; range 5 to 12 mmol/L).

Patients with no large decelerations. Twelve patients had no large decelerations, so that measurements of episodes of low and high FHR variation could be made until delivery. Seven (58%) patients had received epidural analgesia (compared with 65% of the 136 patients). The mean duration of the first stage (8.3 ± 1 hour) was similar to that of the whole group; that of the second stage was a little longer (89 ± 16.7 minutes). In eight of the patients, episodes of low and high FHR variation continued until delivery; in the other four, long episodes of high FHR variation continued through the second stage of labor. Hence, there is a case for believing that fetal sleep states continue throughout labor, as suggested by Spencer and Johnson.[8]

FHR variation in the last hour of labor and base deficit at delivery. If episodic cycles of FHR variation persist in labor randomly with time, then we should expect that there would be no correlation between FHR variation and the fetal acid-base status at delivery. There were 125 (92%) patients in whom the last hour of labor was complete (<10% missing), with long-term FHR variation (mean ± SE) 48.3 ± 1.85 milliseconds (range 18 to 155 milliseconds) and mean arterial base deficit 8.7 ± 0.39 mmol/L (range 1 to 26 mmol/L). There was no significant correlation between the FHR variation averaged over the last hour of labor and the umbilical arterial base deficit at delivery ($r = 0.04$).

Comment

The most important conclusions from these observations are that (1) measured episodic FHR variation continues in labor; (2) the amplitude of FHR variation in episodes of low variation overlaps that predictive of abnormal antenatal fetal outcome; and (3) no significant correlation between long-term FHR variation and base deficit on delivery exists.

Before birth, very low FHR variation (short-term <2.5 milliseconds; long-term <12.5 milliseconds or <4.2 beats/min) maintained for 1 hour is diagnostic of impending death in fetuses who are growth retarded and chronically hypoxemic.[3] Fetuses with such gross and prolonged reduction of FHR variation are occasionally encountered in early labor, with an incidence of <1 in 500. In another category of fetus, FHR variation falls rapidly and abnormally in association with a terminal decline in basal rate in gross end-stage bradycardia. Excluding these two rare events, we must consider the circumstances in which FHR variation falls in labor to limits that may be predictively hazardous. Fetal rest-activity cycles were described >10 years ago[10] and persist in labor, but the implications for FHR analysis have not been faced. The concept of a smooth FHR trace preceding fetal death in utero derives from earlier studies associated with the introduction of "electronic evaluation of the fetal heart rate" by Hon[11] and others.[12-14] However, in some of these studies the patients had received diazepam, the effects of which on FHR were not appreciated at that time. In other studies, there were no records of whether mothers were given drugs, nor were there records of whether the duration of low FHR variation was of recent origin or might have preceded the onset of labor; it is impossible to be sure that the fetuses were not already compromised before labor.

It has been too readily assumed that acute fetal hypoxemia causes a prolonged reduction of FHR variation. The evidence is indirect and slight. In both sheep[5] and rhesus monkeys[15] acute hypoxemia causes an increase in FHR variation. For obvious reasons, no such direct experiments have been made in human beings. Growth-retarded and chronically hypoxemic human fetuses have reduced FHR variation before birth, yet when the PaO_2 is rapidly increased at delivery, FHR variation is not restored to normal levels at once.[16] There are alternative explanations of the reduced FHR variation in chronic hypoxemia associated with growth retardation that have not yet been tested; for example, the variation could be because of the reduced amino acid supply or altered fetal metabolism. The mechanism is unknown.

This study differs from that of Spencer and Johnson[8] in design and in some results. First, measurements were made with the aid of computers, not by visual analysis, and mostly during the early part of the first stage of labor. Our measurements were independent of observer variation, which has been found to be significant.[17] In Spencer and Johnson's identification of behavioral cycles, observer variability amounted to 11%. Second, we measured the amplitude of FHR variation. The results were similar in several respects. Thus we all concluded that there were well-defined episodes of low and high FHR variation in the first stage of labor similar to those observed before birth. Likewise, there was no evidence of diurnal variation.

The duration (mean ± SE) of episodes of low FHR variation was no different (24.3 ± 1.3 minutes) from Spencer and Johnson's[8] measurements (25 ± 0.4 minutes; SE calculated from their Table II). Although the duration of episodes of high variation (45.1 ± 2.7 minutes) in our study was less than in theirs (66 ± 2.5 minutes), the frequency distribution is skewed and the computer picks up brief, easily overlooked episodes. We found a decline in the duration of episodes of high FHR variation with time. Therefore our lower values cannot be attributed to selection of the earlier part of the first stage of labor; Spencer and Johnson included the entire first stage. Our results agree with the durations of fetal behavioral states (median values in 1F 17.5 minutes, in 2F 46 minutes), estimated by measuring fetal body, breathing, and eye movements and by observing FHR variation, before birth at 38 weeks' gestation.[18]

A more important difference is in the incidence of successive episodes of FHR cycling, which according to Spencer and Johnson[8] is less often present in spontaneous labor (38%) as compared with induced labor (68%), but only significantly different when the record length is shorter. Our results showed an identical incidence in spontaneous and induced labor. Our criteria were computer analysis and the presence of at least one completed cycle; Spencer and Johnson used visual analysis and required two cycles before recognizing the presence of cycling. Our patients had a higher incidence of epidural analgesia in spontaneous labor, but that would have been expected to reduce cyclic behavioral activity in the fetus, if it had any effect at all. Also, whereas Spencer and Johnson concluded that 48% of all cardiotocograms with evidence of behavioral cycling had periods when FHR variation was <5 beats/min, we found a lower incidence.

We conclude that episodic changes in FHR variation normally persist in labor, and we speculate that such changes may modify the pattern of decelerations. The allegation that an episode of low FHR variation always indicates danger needs reconsideration.

We thank many midwives and clinical colleagues for their help with the collection of data.

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Ultrasonographic diagnosis of symptomatic deep venous thrombosis in pregnancy

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Color flow imaging combined with compression ultrasonography were used to prospectively evaluate symptomatic patients first seen with suspected deep vein thrombosis during pregnancy. The incidence of symptomatic deep vein thrombosis (7 of 10,000 deliveries) shown prospectively by ultrasonography was similar to that of historic controls who had a diagnosis confirmed by venography (11 of 26,191 deliveries). A preferential pattern of proximal left-sided iliac or femoral vein involvement was common to both cohorts, being seen in five of the seven cases diagnosed by ultrasonography and eight of the eleven cases diagnosed by venography. We conclude that ultrasonography, consisting of compression ultrasonography and color flow imaging, can replace venography in the diagnosis and evaluation of the extent of symptomatic deep vein thrombosis during pregnancy. (Am J Obstet Gynecol 1991;165:625-9.)

Key words: Venous thrombosis, ultrasonography, pregnancy

Pregnancy is a well-recognized risk factor in the development of deep venous thrombosis in women younger than 40 years of age.[1] This diagnosis has until recently been made on clinical grounds,[1, 2] by venography,[3] or with the use of impedance plethysmography.[4-5]

Compression ultrasonography has been shown to have an accuracy >95% for the diagnosis of venous thrombi located within the femoral and popliteal venous segments.[6-9] Although it might be possible to infer that the same accuracy is attained in the pregnant patient, this issue has only been addressed in part in a report of three cases.[10]

This study proposes to evaluate the utility of color flow imaging and compression ultrasonography in the diagnosis and evaluation of the extent of symptomatic deep vein thrombosis in the antepartum patient.

Material and methods

Prospective cohort. Antepartum patients first seen with clinical suspected symptomatic lower extremity venous thrombosis were prospectively studied between March 1988 and March 1989. During this time interval, 10,000 deliveries were performed at our institution. There were 28 patients who ranged in age from 17 to 40 years (mean, 26.8 years ± 6.1 SD). When first seen, patients were on average 26.4 ± 9.5 weeks into their pregnancy for a range of 6 to 39 weeks. All complained of leg swelling, eight had additional complaints of calf pain, and eight localized the pain to the thigh.

Retrospective cohort. The medical records and venograms of all antepartum patients identified retrospectively as having had venography performed between August 1984 and April 1987 were reviewed according to institutional guidelines. Twenty-two such cases were identified. The number of deliveries during this time period was 26,191. The average age of this group was 24.4 years ± 7.0 SD (range, 18 to 41 years). When first seen, these patients were on average 25 ± 8.1 weeks into their pregnancy for a range of 8 to 38 weeks.

Nonpregnant cohort. Between October 1988 and March 1989, 51 nonpregnant patients had both ultrasonography and venography performed because of the clinical suspicion of deep vein thrombosis of the lower extremity. There were 23 males and 28 females with an average age of 54.8 years ± 16.8 SD (range, 18 to 89 years). Forty-three patients were referred because of symptoms, whereas eight were undergoing postoperative surveillance for the presence of deep vein thrombosis. There were 39 inpatients and 12 outpatients.

Techniques. Ultrasonography was performed with the aid of a 5 MHz linear array transducer (Acuson, Computed Sonography, Mountain View, Calif.). The imaging protocol consisted of using distention of the common femoral vein in response to the Valsalva maneuver to exclude obstructing thrombus in the iliac veins.[11] The remainder of the femoral popliteal venous system was then surveyed with color Doppler flow imaging with flow augmentation in the femoral and popliteal vein to exclude nonobstructing thrombosis. Compression ultrasonography was also performed

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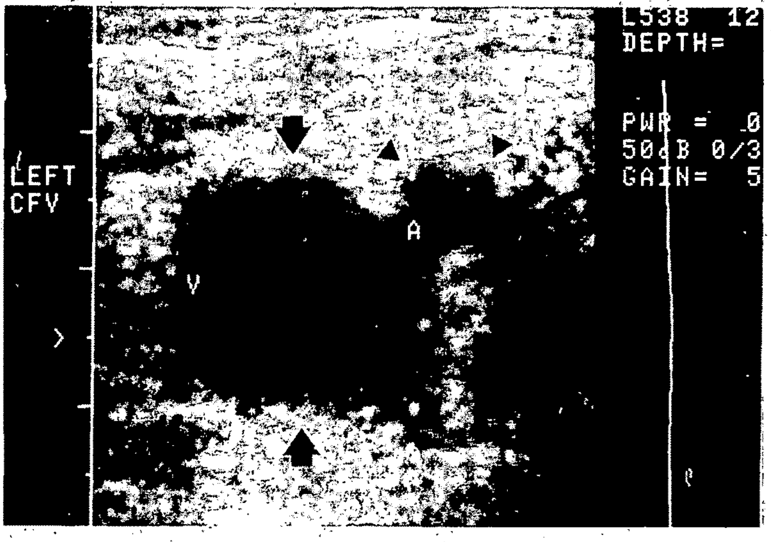

Fig. 1A. This transverse view of the left common femoral vein shows a distended vein *(arrows)* medial to the artery *(arrowheads)*.

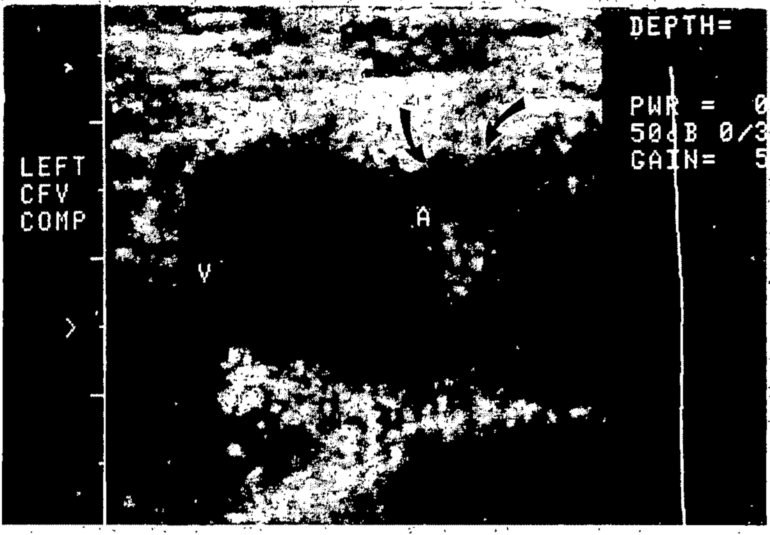

Fig. 1B. Progressive compression causes deformation of the artery *(curved arrow)* before the vein. This loss of compressibility in spite of the lack of echogenic signals within the vein is diagnostic of venous thrombosis.

along the course of the femoral and popliteal veins with the transducer held transverse to the vein. All patients were examined in the supine position. The popliteal vein was imaged with the extremity in slight external rotation or froglike position. Deep vein thrombosis was diagnosed when a venous segment was noncompressible and did not show any flow signals. If the patient manifested localized symptoms a survey was also performed at the site of symptoms, whether it be in the calf or in the thigh.[12]

Venography. Venography was performed with a modification of the approach described by Rabinov and Paulin[13] with a 23 or 25 gauge needle used to cannulate a dorsal pedal vein and dilute contrast material injected under fluoroscopic guidance.

Data analysis. Comparison of the distribution of venous thrombi was made using χ^2 statistics. Proximal venous thrombosis refers to either iliac, common femoral, or superficial femoral vein involvement with sparing of the popliteal veins. Distal venous thrombosis is limited to the calf and popliteal veins with sparing of either the iliac, common femoral, or superficial femoral veins.

Sensitivity and specificity were determined according to recommendations made by McNeil et al.[14] The sensitivity and specificity of the ultrasonographic imaging

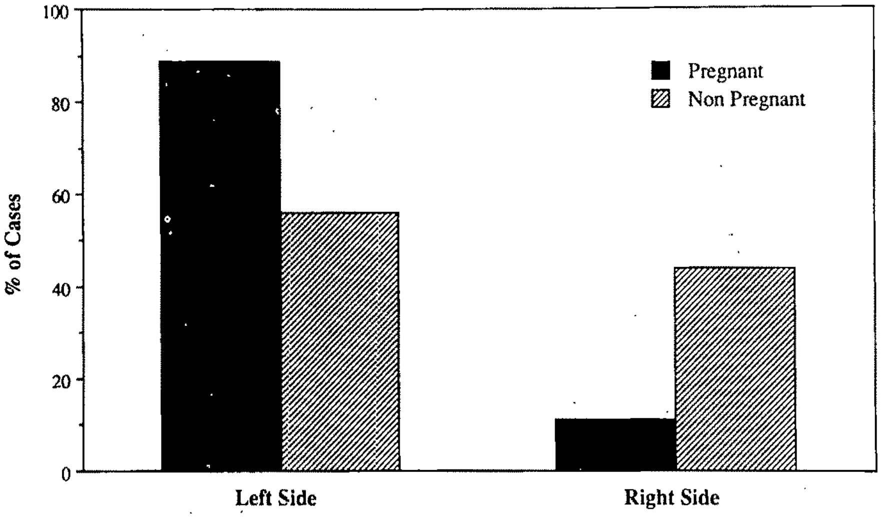

Fig. 2. The figure depicts the affected side of venous thrombosis in the patients studied. Comparison of the side involved by deep vein thrombosis in pregnant and nonpregnant patients shows the preferential left-sided involvement during pregnancy.

protocol was evaluated by comparison to the phlebographic results in the nonpregnant patients because none of the pregnant patients with ultrasonographic examinations underwent additional phlebography.

Results

Pregnant subjects. In the retrospective cohort, there were 22 patients in whom venography was performed out of 26,191 pregnancies at risk. In 11 of these, the diagnosis of deep venous thrombosis was confirmed. Thrombosis of the lower popliteal veins was noted in three, whereas proximal thrombus sparing the popliteal vein but involving the iliofemoral system was noted in the other eight. The left side was involved in all 11 cases. Venous thrombosis occurred during the first ($n = 2$), second ($n = 5$), and third ($n = 4$) trimesters.

In the prospective cohort diagnosed by ultrasonography, 28 subjects were suspected of having deep venous thrombosis. The diagnosis was made in seven instances by noting loss of compressibility and the absence of flow within the involved segment. Proximal venous thrombosis involving the iliac and femoral vein but sparing the popliteal vein was diagnosed in five of these (Figs. 1A and 1B). The left side was involved in five of the seven cases. Deep vein thrombosis occurred during the first ($n = 2$), second ($n = 1$), and third ($n = 4$) trimesters. All patients diagnosed by ultrasonography or venography were treated with heparin and went on to be delivered of infants. No cases of clinically evident pulmonary emboli were reported.

Nonpregnant subjects. A total of 45 of 51 venograms were judged to be diagnostic with three being technically limited and three nondiagnostic.

There were 13 instances of negative venograms; all 13 had a normal noninvasive ultrasonographic examination. Three of seven cases of below-knee thrombosis were prospectively detected. The 25 instances of femoropopliteal venous thrombosis were diagnosed prospectively by ultrasonography. There was a slight preferential left lower extremity involvement (14/25). Distal thrombosis involving the popliteal and femoral veins but sparing the iliac veins was seen in 22 of 25 cases. Proximal thrombosis limited to the iliofemoral veins with sparing of the popliteal veins was seen in three patients with pelvic malignancies.

Pregnant vs nonpregnant subjects. Preferential left-sided involvement (Fig. 2) was more common in the pregnant population (16/18 vs 18/32; $\chi^2 = 4.24$; $p < 0.05$). Proximal iliofemoral thrombosis was more common in the pregnant population (Fig. 3) as compared with the nonpregnant population (13/18 vs 3/32; $\chi^2 = 18.1$; $p < 0.001$).

Comment

This study confirms that the incidence of symptomatic deep venous thrombosis during pregnancy is low and similar to that cited in previous reports (i.e., between 0.4 to 0.7/1000 deliveries).[3, 4] Among subjects younger than 40 years of age, pregnancy is a major risk factor for venous thrombosis and pulmonary embolism.[1] Once recognized, venous thrombosis is treated with judicious anticoagulation.[15, 16] The use of heparin is favored, in spite of the logistical difficulties of its administration, because of the possible teratogenic effects of sodium warfarin (Coumadin).

A previous report of three cases suggested that venous ultrasonography could be used to make the diagnosis of deep vein thrombosis during pregnancy.[10]

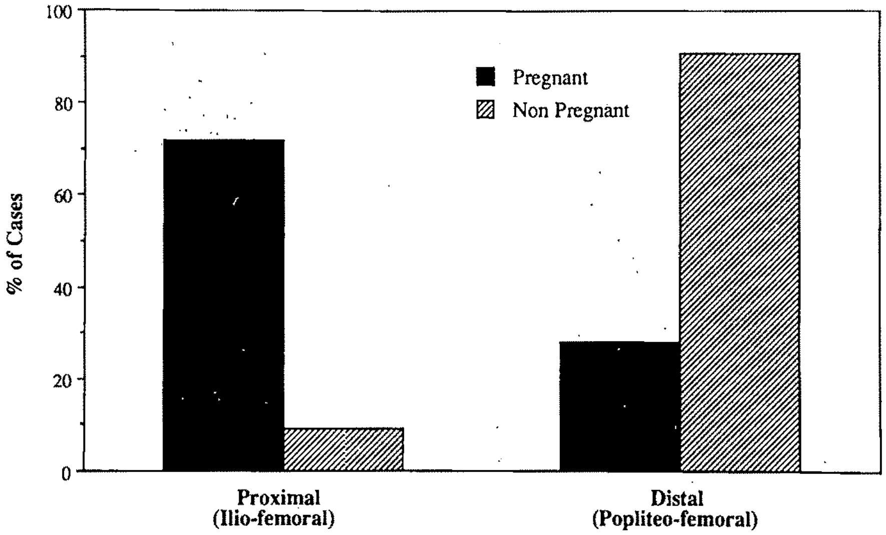

Fig. 3. This figure shows the distribution of venous thrombosis in the leg veins. Proximal iliofemoral involvement with popliteal vein sparing is more likely during pregnancy.

This study examines the utility of ultrasonography in a larger number of patients. A side-by-side comparison with venography was difficult to justify because of the risks of contrast material and radiographs to both mother and fetus. The latter is important inasmuch as the fetus is exposed to radiation from radiographs taken of the upper thigh and pelvis. However, these films are important because this and previous studies have shown preferential iliac vein involvement with downward spread of thrombosis during pregnancy. We therefore studied the diagnostic utility of ultrasonography in two ways. The first was to confirm that during pregnancy the pattern of deep venous thrombosis seen prospectively with ultrasonography is similar to that seen when venography is performed. The historic population was made up of all pregnant patients suspected of having symptomatic deep venous thrombosis from among 26,191 consecutive pregnancies. The clinical suspicion of venous thrombosis led to venography. The prevalence of deep venous thrombosis in this group was 50%. This high proportion of positive venograms suggests a clinical hesitation in performing phlebography during pregnancy. In this prospective cohort, venous ultrasonography showed a similar distribution of venous thrombus. The number of ultrasonographic examinations performed in this group (28/10,000) is higher than that for the patients who had venography (22/26,191) and suggests that the diagnostic threshold for performing the test is lower. This possibility is supported by the lower proportion of positive examinations (25%) in the group diagnosed by ultrasonography. Both groups did, however, show a similar preferential left-sided involvement of venous thrombosis. The pathophysiologic mechanism responsible for this phenomenon is believed to be increased stasis because of the crossing of the iliac vein beneath either a low bifurcating aorta or the right iliac artery.[17] Besides a simple compressive effect, there is also a higher incidence of venous webs forming at this site.[14]

Color Doppler ultrasonography and compression ultrasonography were compared with venography in nonpregnant patients not only to verify the overall accuracy of the technique but also to establish the pattern of involvement in the nonpregnant population. Although preferential involvement of the iliac and proximal femoral veins is common in the pregnant patient, it is uncommon in the nonpregnant group.[19, 20]

In a recent retrospective review, Becker et al.[21] show the sensitivity and specificity of ultrasonography for femoropopliteal system thrombosis to be 96% and 99%, respectively. When calf thrombi are included, the diagnostic sensitivity of the test decreases. This finding may not apply to future studies that will focus on visualization of the paired perineotibial and muscular veins of the calf.[12] The use of anticoagulation for calf vein thrombosis, already controversial in the nonpregnant patient, is in fact harder to justify during pregnancy.

The addition of color Doppler imaging to compression ultrasonography is considered to be supportive in nature. In none of these instances was the diagnosis of deep venous thrombosis made without loss of compressibility being present. In fact poor venous flow was often seen during the third trimester with the patient supine and resolved in the opposite decubitus position in all cases. Color Doppler ultrasonography by itself could have led to false-positive diagnoses.

We therefore conclude that color Doppler ultrasonography and compression ultrasonography can be used to make the diagnosis of deep venous thrombosis of the lower extremity during pregnancy and to effectively replace venography.

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Treatment of fetal supraventricular tachycardia with flecainide acetate after digoxin failure

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Transplacentally administered digoxin is the drug of choice for the treatment of fetal supraventicular tachycardia. We describe a case of fetal supraventricular tachycardia associated with fetal hydrops that did not respond to digoxin treatment because of a lack of transplacental passage. In contrast, flecainide acetate crossed the placenta and cured the fetus. The clinical implications of this new treatment are discussed. (Am J Obstet Gynecol 1991;165:630-1.)

Key words: Flecainide acetate, digoxin, fetal tachycardia

The treatment of fetal supraventricular tachycardia has been a difficult task. The most commonly used drug is digoxin alone or in combination with propranolol or verapamil. We describe a case of supraventricular tachycardia unresponsive to digoxin that was successfully treated with flecainide acetate.

Case report

A 22-year-old, gravida 1, para 0 woman was referred to the York Hospital antenatal testing unit at 31 weeks' gestation for fetal arrhythmia. Real-time ultrasound (Toshiba 270 a-sonolayer, Toshiba America Medical Systems, Tustin, Calif.) revealed mild hydramnios and fetal ascites. Fetal M-mode echocardiography revealed fetal supraventricular tachycardia at a rate of 240 beats/min with no evidence of atrioventricular block. Two-dimensional echocardiography revealed a structurally normal fetal heart with moderate dilatation. Duplex pulsed-wave and color Doppler ultrasonography revealed tricuspid regurgitation and reverse flow in the inferior vena cava. Myocardial contractility was decreased with a right ventricular fractional shortening consistently <20%. Treatment was initiated with digoxin transplacentally and 48 hours later the fetal heart rate (FHR) was 204 beats/min in spite of a maternal serum level of 2.1 ng/ml. Fetal umbilical vein level was 0.9 ng/ml (therapeutic, 0.9 to 2.0 ng/ml). Flecainide acetate was started at a dosage of 100 mg orally every 8 hours. Over the next 96 hours FHR converted to normal sinus rhythm of 120 beats/min. Maternal flecainide trough levels drawn at 24 hours, 48 hours, and 96 hours after initiation of treatment were 0.5, 0.6, and 0.8 μg/ml (therapeutic level, 0.2 to 1 μg/ml). No maternal side effects were noted and daily maternal electrocardiogram for 3 days was normal. At 56 hours after conversion a decision was made to discontinue both the digoxin and flecainide because of nonreactive nonstress test (NST) results, which gradually became reactive after discontinuation of flecainide.

Approximately 36 hours after discontinuation of treatment the FHR returned to pretreatment levels. Flecainide acetate 150 mg was given orally alone and 30 minutes later the FHR converted to normal. The patient received an additional 150 mg in 12 hours and every 12 hours thereafter. After 4 days at a stable heart rate of 120 beats/min with nonreactive NST results, flecainide was gradually decreased by 50 mg decrements to 50 mg every 12 hours, and over this period of time the NST results became reactive with normal beat-to-beat variability. Maternal serum flecainide trough level during this period was 0.2 μg/ml.

Right ventricular function improved, inferior vena cava flow pattern returned to normal, and cardiac dimensions became normal within the first 20 days. The placental thickness declined from 6.4 to 4.2 cm and fetal ascites resolved completely in 10 days.

The patient was delivered vaginally at 41 weeks' gestation. Maternal and fetal serum trough levels of flecainide at the time of delivery were 0.2 and 0.1 μg/ml, respectively. The Apgar scores were 9 and 10 at 1 and 5 minutes, respectively, and the venous blood gas values were normal. Birth weight was 3480 gm. Postnatal echocardiography was normal. At 48 hours after birth the neonate was started on prophylactic digoxin.

Comment

Digoxin has been the drug of choice and its safety is recognized. However, digoxin alone is not successful in all cases and this most likely is a result of poor transplacental passage.[1] Flecainide has been used successfully in the treatment of children with tachyarrhythmia resistant to conventional treatment and was found to

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be safe.[2] Flecainide treatment of fetal supraventricular tachycardia by intravenous administration to the mother resulted in conversion of the fetal arrhythmia to regular sinus rhythm in less than 10 minutes.[3] In this case, toxic maternal serum levels of digoxin (2.1 ng/ml) resulted in a fetal serum level of 0.9 ng/ml, which is at the lowest end of the therapeutic range. The addition of propranolol was rejected because of the theoretic risk of intrauterine growth retardation and the addition of verapamil was rejected because of its association with fetal and neonatal deaths. Direct intramuscular fetal injections were considered unsafe in this patient. Flecainide acetate was then chosen because it has been shown to pass across the placenta rather easily with fetal levels as high as 86% of maternal serum levels.

Flecainide acetate in this case has been associated with nonreactive NST results and poor beat-to-beat variability, a dosage-related event that subsides spontaneously with the lowering of the dosage. Surveillance for fetal well-being was performed by means of biophysical profile when the beat-to-beat variability was poor and with twice-weekly NSTs when the beat-to-beat variability returned to normal. Fetal blood sampling for determination of digoxin level was instrumental in defining the reason for the failure of digoxin to control the tachycardia. We conclude that the use of flecainide acetate for the treatment of supraventricular tachycardia may be a safe and successful alternative for arrhythmias resistant to digoxin.

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Antimicrobial therapy in preterm premature rupture of membranes: Results of a prospective, double-blind, placebo-controlled trial of erythromycin

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This study was conducted to evaluate the effectiveness of oral erythromycin treatment in safely prolonging pregnancy among women experiencing preterm premature rupture of membranes. Sixty-five women were randomly assigned to receive double-blind treatment with either erythromycin base or an identical-appearing placebo three times daily for 7 days. Only women between 23 and 34 completed weeks' gestation who did not have an indication for delivery were enrolled in the study. Pretreatment microbiologic tests were obtained and women were followed expectantly. Fifty-five women and their newborns completed the protocol and were fully evaluated. Overall, time from rupture of membranes to onset of labor and to delivery was longer, although not significantly, for erythromycin-treated women. Similarly, there was a trend for reduced neonatal intensive care (level II, $p = 0.07$). When gestational age at enrollment was controlled, erythromycin treatment of women between 28 to 32 weeks' gestation was associated with a prolonged interval from enrollment to delivery [erythromycin: 292 hours (5 to 679); placebo: 54 (12 to 323); $p < 0.044$]. Fifty percent of erythromycin-treated women between 28 to 32 weeks' gestation continued their pregnancies at least 13 days after premature rupture of membranes, whereas 50% of placebo-treated women were delivered of infants within 4 days ($p = 0.02$). Erythromycin treatment among women <28 and between 33 to 34 weeks' gestation was not associated with prolonged latency or other changes. There were no differences between erythromycin- and placebo-treated women in the occurrence of clinically recognized chorioamnionitis, postpartum endometritis, or neonatal infectious morbidity. In this double-blind, placebo-controlled trial, erythromycin treatment was well tolerated, safe, and associated with prolongation of pregnancy and reduced intensive neonatal care requirements for selected mother-newborn pairs with preterm premature rupture of membranes. (AM J OBSTET GYNECOL 1991;165:632-40.)

Key words: Premature rupture of membranes, treatment, antibiotic

Rupture of fetal membranes before the onset of labor and before completion of 37 weeks' gestation occurs among 1% to 2% of pregnancies and is directly related to 30% to 40% of preterm births.[1, 2] Optimal management of patients with preterm premature rupture of membranes (PROM) has not been defined, but requires balancing risks of prematurity versus those of maternal and perinatal infection.[3] Benefits of improved care for women with preterm PROM may be substantial. Goldenberg[4] calculates that an infant's chance of surviving without major handicap increases up to 3% each day between 23 and 30 weeks' gestation, regardless of membrane status.

Although the pathogenesis of preterm amniorrhexis remains incompletely understood, considerable information indicates that infection and inflammation within the amniochorion or the adjacent decidua likely precede some instances of preterm PROM.[5-8] Alternatively, ascending intrauterine infection after preterm PROM is a recognized cause of maternal and perinatal infectious morbidity. If infection is indeed a cause or contributing factor in preterm PROM as well as a complication that develops after rupture of membranes and hastens the eventual onset of labor, it is possible that appropriate antimicrobial therapy may benefit some women and their infants.[3, 9-13]

Therefore a prospective, randomized, double-blind, placebo-controlled study was undertaken to evaluate the efficacy of a short course of oral erythromycin to safely prolong gestation, reduce risks of maternal and perinatal infectious morbidity, and further define clinical and microbiologic circumstances in which erythromycin treatment may be beneficial after preterm PROM.

Methods

Between July 1986 and June 1988, 154 women were admitted to University Hospital, Denver, with rupture of membranes before 35 weeks' gestation. Rupture of

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membranes was clinically documented by the presence of vaginal pooling of amniotic fluid or visualization of amniotic fluid released from the cervical os as well as observation of a ferning pattern on microscopic examination of a dried smear obtained from the vaginal pool or detection of alkaline pH with Nitrazine paper (E. R. Squibb and Sons, New Brunswick, N.J.). Women with singleton pregnancies and clinically documented rupture of membranes between 23 and 34 completed weeks' gestation were evaluated for study enrollment. Exclusion criteria included possible labor (demonstrated two or more regular uterine contractions every 10 minutes persisting after observation and intravenous hydration), presence of a maternal or fetal indication to facilitate labor or delivery (including evidence of chorioamnionitis, fetal distress, prolapsed cord, pregnancy-induced hypertension, abruptio placentae), placenta previa, cervical cerclage, known infectious process requiring antimicrobial therapy, use of vaginal or systemic antimicrobial agents in the preceding 2 weeks, presence of known uterine or fetal anomalies, history of vaginal bleeding within the prior month, serious maternal medical complications (such as diabetes mellitus, severe cardiac, kidney, liver, respiratory, or immunologic disease), or history of allergy or intolerance to erythromycin. Eighty-four women met study inclusion criteria, and 65 (66%) of the eligible women were successfully enrolled in the study protocol. Each participant signed written informed consent approved by the University of Colorado Health Sciences Center Human Subjects Committee. Informed consent could not be obtained from 19 women.

Ten women were subsequently excluded from analysis before breaking the treatment code. In four of these women, rupture of membranes was excluded by amniocentesis with injection of indigo carmine dye; all these women were delivered of infants at term. Two women with minimal or no prenatal care and poor dating criteria were excluded inasmuch as they entered labor and were delivered of term infants after one or two doses of study treatment. Labor was induced for one patient when pregnancy-induced hypertension was noted 1 day after enrollment. This infant was subsequently found to have trisomy 18 and died. One patient with rupture of membranes at 23 weeks' gestation was lost to follow-up after she left the hospital to return to her home state against medical advice. One patient, enrolled at 26 weeks' gestation with suspected membrane rupture since 18 weeks' gestation, was delivered of an infant with hypoplastic lungs and limb contractures who died within 48 hours of birth. Examination of the placenta revealed a partial molar pregnancy. The final patient was excluded because of a pharmacy error. The total analyzed sample included 55 women and their newborns.

Clinical evaluation. On enrollment, women were interviewed to obtain demographic, social, behavioral, medical, and obstetric information. During the initial sterile speculum examination, which documented rupture of membranes, microbiologic samples were obtained as follows. The anterior vaginal fornix and lateral vaginal wall, away from the cervix, were swabbed with sterile cotton-tipped swabs. One of these samples was rolled onto a slide and allowed to air-dry for Gram's stain evaluation for bacterial vaginosis and *Mobiluncus* species morphotypes with previously described criteria.[14] Material on a second swab was combined with a drop of 10% potassium hydroxide on a slide to test for release of amine odor (whiff test) and examined microscopically for yeast pseudohyphae. Another swab was combined with a drop of saline solution and examined microscopically for motile trichomonads.

Microbiologic methods. Cervicovaginal swabs were inoculated at the bedside onto 5% sheep blood agar (tryptic soy base, Remel, Denver) for recovery of group A and group B streptococcus, *Staphylococcus aureus*, *Gardnerella vaginalis*, and yeast spp. Additional vaginal swabs were inoculated into a standard mycoplasma transport medium for recovery of *Mycoplasma hominis* and *Ureaplasma urealyticum*. Separate endocervical swabs were immediately inoculated onto a chocolate agar and a modified Thayer-Martin medium split plate for recovery of *Neisseria gonorrhoeae*, and into sucrose-phosphate glutamate transport media for recovery of *Chlamydia trachomatis*.

Microorganisms were identified with standard techniques.[15] Cultures for *M. hominis*, *U. urealyticum*, and *C. trachomatis* were frozen at − 70° C and processed in batches. The presence of chlamydia was determined by combined technique. After 48 hours incubation at 35° C, inoculated cycloheximide-treated McCoy cell monolayers were treated with fluorescein-conjugated antichlamydial monoclonal antibody (Syva Microtrak, Syva Co., Palo Alto, Calif.) and examined microscopically for the presence of inclusions.[16] Negative cultures were not processed further. For recovery of *M. hominis* and *U. urealyticum*, a modified commercially available agar (Mycotrim GU, Hana Biologics, Inc., Alameda, Calif.) was inoculated with 1 ml of thawed transport media and processed according to established techniques. Posttreatment cultures were not obtained.

Study treatment. Study patients were randomly assigned to receive either erythromycin base 333 mg (E-Mycin, Upjohn Co., Kalamazoo, Mich.) orally three times daily or an identical-appearing placebo. Erythromycin and placebo tablets were double-blinded and distributed in sequentially numbered, identical bottles provided by The Upjohn Company, Kalamazoo, Mich. Study treatment continued until the patient entered active labor or for a total of 7 days. Treatment was randomized according to a computer-generated random number list. A copy of the randomization code was sealed by The Upjohn Company and kept in the University Hospital pharmacy for the duration of the study.

Women were hospitalized and managed expectantly with bed rest and bathroom privileges while being observed for evidence of continued fetal well-being, infection, or the onset of labor. Patients were examined twice daily for evidence of uterine tenderness and the presence, as well as odor and color, of amniotic fluid. Maternal temperature and pulse and fetal heart rate were determined every 4 hours until onset of labor. An external fetal monitor tracing was obtained daily to evaluate fetal heart rate parameters and to detect uterine contractions. Maternal white blood cell counts were performed every other day. Corticosteroids were not administered, and only two women received single doses of subcutaneous terbutaline before transport. Women found to be vaginally colonized with group B streptococcus were given appropriate antibiotic treatment, usually intravenous ampicillin in labor. Women with positive endocervical cultures for *C. trachomatis* were given appropriate antimicrobial treatment after delivery. Neonatal care providers were notified at delivery or as soon as results were available when either group B streptococcus or *C. trachomatis* were recovered from the mothers. Women with findings of urinary tract infection or significant bacteriuria were treated with a course of oral nitrofurodantoin. Follow-up information was obtained by review of maternal and newborn medical records 6 and 12 weeks after delivery, respectively.

Definitions. Gestational age was determined before data analysis and was based on overall best obstetric criteria that included last menstrual period, antenatal record of fetal growth, detection of fetal heart rate with fetoscope, ultrasonographic measurements, and neonatal gestational age assessment.[17] The latent period was defined as time from rupture of membranes to delivery. The diagnosis of chorioamnionitis was determined on the basis of clinical criteria of maternal fever or at least two of the following: tachycardia, uterine tenderness, foul amniotic fluid, fetal tachycardia, with or without maternal leukocytosis.

Data analysis. Sample size calculation performed before the study determined that a sample with 30 patients in each arm provided a 70% chance of detecting a 30% increase in the number of patients who continued their pregnancies for at least 1 week, with α set at 0.05. Data were summarized with descriptive statistics. Group differences for noncontinuous data were tested for significance with χ^2 or Fischer's exact tests (two-tailed). Continuous data were analyzed with the Student *t* test. Group differences for the time interval between rupture of membranes and entry into study and delivery were compared with survival analysis (SAS Lifetest procedure) log rank test or Wilcoxon rank sum test. A p value <0.05 was considered significant.

Results

Twenty-eight women were randomized to receive erythromycin and 27 received placebo. Demographic, historic, obstetric, and behavioral characteristics were similar between treatment groups (Table I). Ten (18.2%) of the enrolled women had completed <28 weeks' gestation; 24 (43.6%) were between 28 and 32 weeks' gestation, and 21 (38.2%) were >32 weeks' gestation at enrollment. Equivalent numbers of women in each treatment group received digital examination to assess cervical dilatation after rupture of membranes and before onset of the study. Multiple other demographic and historic factors (not presented) did not differ among study patients.

Risk factors associated with prematurity. Group differences for patients who received erythromycin or placebo for maternal and antenatal risk factors associated with prematurity are also shown in Table I. Five (17.9%) patients in the erythromycin group reported recreational drug use: one woman reported using intravenous heroin occasionally during early pregnancy; the remaining four patients in the erythromycin group and two (7.4%) patients in the placebo group reported occasional marijuana use. No patient reported using any form of cocaine.

Maternal medical problems were clinically well controlled and included first-trimester cholecystectomy and appendectomy (one patient), hypertension (two patients), depression (one patient), late second-trimester bronchitis and pneumonia (three patients), gestational diabetes (one patient), cholestatic jaundice (one patient), uterine myoma (one patient), and asthma (one patient). One patient in the placebo group was treated for pyelonephritis at 17 weeks' gestation; she was enrolled in the study at 32 weeks' gestation. Subsequent to entering the study, four patients (three erythromycin group, one placebo group) were treated with oral nitrofurodantoin for bacteriuria. Congenital anomalies were detected among three infants whose mothers had received erythromycin (two vessel cord; tracheal esophageal fistula with esophageal atresia, and ventral septal defect; metatarsus adductus) and two infants from the placebo group (two vessel cord and mild hydrocephalus).

Microbiologic findings. Microorganisms recovered at study initiation are listed in Table II. Similar types and numbers of isolates were recovered from each treatment group. *N. gonorrhoeae* was recovered from one erythromycin-treated patient at 28 weeks' gestation; this patient experienced an intrauterine fetal death within 12 hours of hospital admission and within

Table I. Maternal, demographic, historic, behavioral, and antenatal risk factors associated with increased risk of prematurity

	Erythromycin (n = 28)	*Placebo (n = 27)*
Mean maternal age (range)	25.4 ± 6.6 (18-41)	24.2 ± 5.0 (18-38)
Ethnic group		
Northern European	17 (61.0%)	20 (74.0%)
African American	3 (11.0%)	0
Hispanic surname	8 (29.0%)	7 (26.0%)
Married	17 (61.0%)	20 (74.0%)
Education <12 yr	11 (40.7%)	11 (44.0%)
Historic		
Mean gravidity (range)*	3.6 ± 2.6 (1-12)	2.4 ± 1.4 (1-6)
Mean parity (range)	1.6 ± 2.2 (0-9)	1.0 ± 1.1 (0-4)
Prior preterm birth	7 (25.0%)	4 (15.0%)
Prior preterm PROM	1 (4.0%)	2 (8.0%)
Behavioral		
Smoking (any)	17 (40.7%)	14 (52.0%)
Alcohol use	6 (22.2%)	5 (18.5%)
Coitus within 7 days of PROM†	14 (63.6%)	16 (66.6%)
Antenatal		
Maternal medical problems‡	3 (10.7%)	8 (29.6%)
Antenatal care initiated after first trimester	10 (40.0%)	14 (58.3%)
Vaginal bleeding	11 (39.3%)	11 (40.7%)
Antenatal antimicrobial treatment	12 (44.4%)	18 (66.7%)
Antenatal treatment for urinary tract infection§	3 (10.7%)	9 (33.3%)

*p = 0.04.
†Data missing for six patients.
‡p = 0.1.
§p = 0.06.

Table II. Microbial findings for erythromycin- and placebo-treated patients

	Erythromycin (n = 28)		*Placebo (n = 27)*	
	No.	*%*	*No.*	*%*
N. gonorrhoeae	1/28	3.6	0	
C. trachomatis	3/26	11.5	2/24	8.3
M. hominis	10/26	38.5	7/26	26.9
U. urealyticum	23/26	88.5	19/26	73.1
G. vaginalis	6/22	27.3	9/27	33.3
S. aureus	1/22	4.6	5/27	18.5
Group B streptococcus	2/28	7.1	3/27	11.1
Yeast species	1/23	4.4	2/27	7.4
Bacterial vaginosis	6/23	26.1	6/23	26.1

2 hours of a reassuring fetal monitor tracing. At delivery, a tight nuchal cord and foul odor of amniotic fluid were noted; placental pathology showed acute chorioamnionitis. Autopsy was refused.

Two (7.1%) patients in the erythromycin group and three patients (11.1%) in the placebo group had group B streptococcus recovered. One woman in each treatment group labored and delivered infants within 1 day of study enrollment and before return of culture results. Each of the other women with group B streptococcus received suitable intravenous antibiotic treatment, one patient in the erythromycin group received cephalexin and two women in the placebo group received ampicillin before labor and delivery. Each patient was retained in their original group. No instance of group B streptococcal neonatal sepsis occurred.

Women from whom *C. trachomatis*, *S. aureus*, or group B streptococcus were recovered were more likely to be >32 weeks gestation at study enrollment when compared with women without these microorganisms (p = 0.02, relative risk [RR], 3.6, 95% confidence interval [CI], 1.4 to 9.6). Conversely, bacterial vaginosis was identified frequently among women ≤32 weeks' gestation at study enrollment. Although not statistically different, nine (32.1%) women enrolled ≤32 weeks' gestation were identified as having bacterial vaginosis compared with 3 (16.7%) women who were >32 weeks' gestation at enrollment (p = 0.3). Similarly, women

Table III. Pregnancy outcome for erythromycin- and placebo-treated patients

	Erythromycin *($n = 28$)*	*Placebo* *($n = 27$)*
Gestational age at enrollment	30.5 ± 3.5 (22-35)	31.5 ± 2.8 (26-35)*
Gestational age at delivery	31.4 ± 3.4 (24-36)	32.3 ± 2.3 (27-35)
Birth weight	1638.5 ± 530.8 (630-2580)	1741.4 ± 444.0 (890-2560)
Hours from study entry to delivery	202 (3-679)†	57 (7-734)
<28 Wk at enrollment	218 (12-511)	582 (127-734)
28-32 Wk at enrollment	292 (5-679)	54 (12-323)‡
>32 Wk at enrollment	32 (3-76)	53 (7-183)

*$p = 0.08$.
†Median (range).
‡$p < 0.05$.

from whom *U. urealyticum* was recovered were more often enrolled at lower gestational ages (87.5% of women ≤ 32 weeks' were positive for *U. urealyticum* versus 70.0% of women >32 weeks' gestation, $p = 0.2$). These women delivered infants weighing nearly 300 gm less (*U. urealyticum*–positive 1641.4 ± 461.0 gm versus *U. urealyticum*–negative 1937.0 ± 426.3 gm, $p = 0.07$).

Pregnancy outcomes. Table III and Figs. 1 and 2 show selected pregnancy outcomes. All patients delivered infants before 37 weeks' gestation. Overall, time from study entry to delivery was longer, although not significantly, for erythromycin-treated women (Table III). With the use of univariate analysis, these differences were significant for women who continued their pregnancies for at least 10 days (erythromycin: 13/27, 48.2%; placebo: 5/27, 18.5%; $p = 0.04$, RR 2.6, 95% CI 1.1 to 5.9). Survival analysis confirmed these findings (Fig. 1).

In spite of random assignment, women who received erythromycin tended to enter the study at a lower mean gestational age ($p = 0.08$). Because of these findings, data were stratified to control for gestational age at enrollment. Stratification (<28 weeks' gestation, 28 to 32 weeks, and ≥ 33 weeks' gestation) was performed to conform with clinical parameters that are associated with different perinatal outcomes among patients with PROM in our center. Among subjects enrolled <28 weeks' gestation, erythromycin-treated patients ($n = 6$) continued their pregnancies for less time than placebo-treated ($n = 4$) women (erythromycin: median 218, range [12 to 511] hours from study entry to delivery; placebo: median 582, range [127 to 734] hours).

Among women with PROM between 28 to 32 weeks' gestation, mean gestational ages at enrollment were similar (erythromycin: 30.3 ± 1.4 weeks versus placebo: 30.6 ± 1.4 weeks, $p = 0.6$). Erythromycin-treated women ($n = 15$) continued their pregnancies significantly longer (median 291, range [5 to 679] hours from study entry to delivery) than placebo-treated ($n = 9$) women (median 54, range [12 to 323] hours; $p = 0.044$). Survival analysis for this subgroup (28 to 32 weeks' gestation) of women showed that 50% of erythromycin-treated women continued their pregnancies from rupture of membranes at least 13 days, whereas 50% of placebo-treated women delivered infants within 3.8 days (log rank test between groups; $p = 0.02$) (Fig. 2). Newborns of erythromycin-treated women weighed more than those receiving placebo (erythromycin: $x = 1829.3 \pm 449$ gm; placebo: $x = 1624.4 \pm 398$ gm; $p = 0.3$).

Gestational age at enrollment was also similar between study groups for women ≥ 33 to 34 weeks' gestation at enrollment. There were no differences among treated ($n = 7$) or untreated ($n = 14$) women. Each of these women delivered within 10 days of rupture of membranes; more than 50% of both treatment groups delivered infants within 3 days of rupture of membranes.

Intrapartum and postpartum maternal and neonatal complications are shown in Table IV. There were no statistical differences between erythromycin- and placebo-treated mothers for measured outcomes. Chorioamnionitis was clinically diagnosed in seven (25%) erythromycin-treated women and six (22.2%) placebo-treated women. Most of these patients began labor spontaneously; three erythromycin-treated and one placebo-treated patients received oxytocin induction of labor. Postpartum endometritis occurred among two erythromycin- and one placebo-treated patients.

In addition to the erythromycin-treated patient who experienced the intrauterine fetal death, five erythromycin-treated women had infants who died during the neonatal period (Table IV). Each of these women had membrane rupture before 27 weeks' gestation (mean, 24 weeks). Four of these infants weighed between 630 to 870 gm at birth and died of complications of prematurity; the remaining infant (PROM at 26 weeks' gestation) delivered at 29 weeks' gestation (1270 gm) with clinical chorioamnionitis. This infant died of complications of prematurity 12 hours after birth. Conversely, among survivors, newborns of erythromycin-

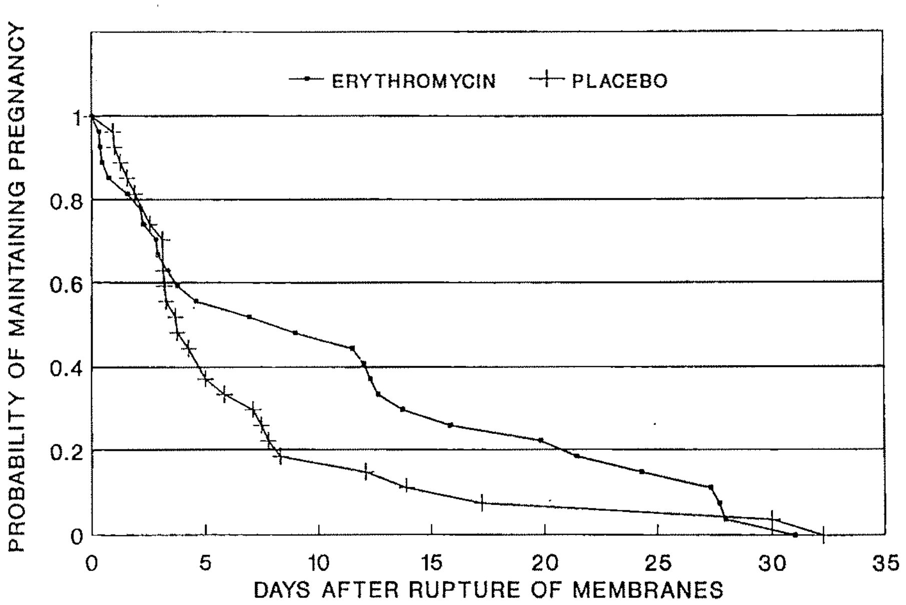

Fig. 1. Estimated risk of delivery after rupture of membranes for erythromycin- and placebo-treated patients. Log rank test between groups $\chi^2 = 0.94$, degree of freedom = 1, $p > 0.1$.

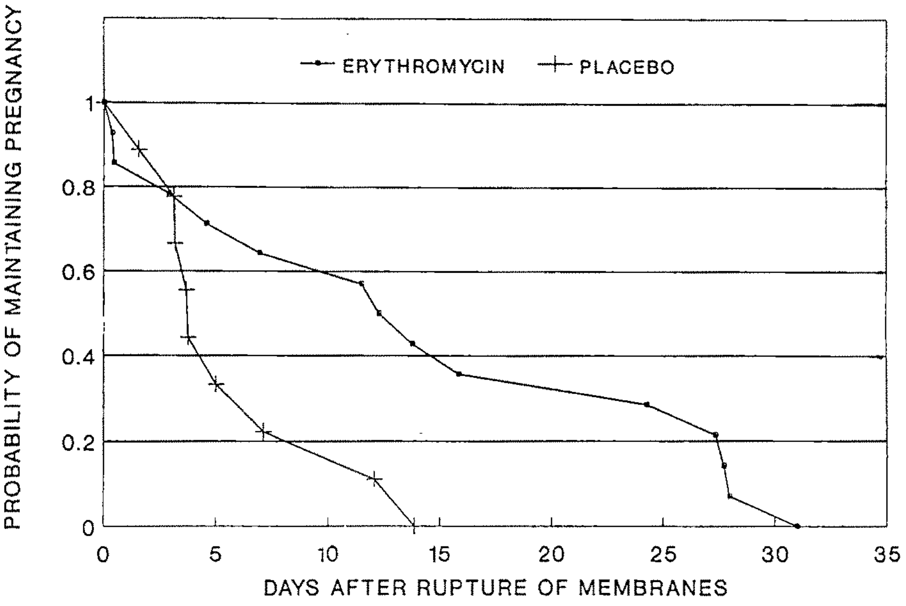

Fig. 2. Estimated risk of delivery after rupture of membranes between 28 to 32 weeks' gestation for erythromycin- and placebo-treated patients. Log rank test between groups $\chi^2 = 5.1$, $p = 0.02$.

treated women required fewer days of level II ($p = 0.07$) nursery care than those of placebo-treated women.

Both erythromycin and placebo were reasonably well tolerated; two patients who received erythromycin noted nausea and vomiting and transient diarrhea developed in one. No other maternal or neonatal sequelae were ascribable to use of erythromycin. No complications were noted among placebo-treated patients. In this relatively small trial, there were no differences for drug versus placebo-treated subjects based on microbiologic findings.

Comment

This study evaluated the efficacy, safety, and tolerance of a short course of oral erythromycin to prolong gestation and reduce the risk of perinatal and maternal morbidity among women with preterm PROM. Overall, erythromycin-treated women showed an increased, but statistically insignificant, time from study enrollment to

Table IV. Selected outcomes and complications among study mothers and newborns

	Erythromycin		*Placebo*	
	No.	%	*No.*	%
Clinical chorioamnionitis	7/28	25.0	6/27	22.2
Birth weight (gm)				
<2000	22/28	78.6	18/27	66.7
<1500	12/28	42.9	8/27	29.6
<1000	4/28	14.3	2/27	7.4
Size small for gestational age	1/28	3.6	3/27	11.1
Fetal or neonatal death*	6/28	21.4	0	0
Treated for possible sepsis	22/26	84.6	27/27	100
Necrotizing enterocolitis	2/26	7.7	4/27	14.8
Intercranial hemorrhage*	5/26	19.2	1/27	3.7
Respiratory distress syndrome	15/26	57.7	15/25	60.0
Pneumonia at birth	1/24	4.2	1/27	3.7
Conjunctivitis	2/24	8.3	4/27	14.8
Mean newborn hospital stay				
Level III	9.5 ± 10.5 (0-46)		14.5 ± 18.9 (0-63)	
Level II	9.0 ± 9.9 (0-30)†		16.5 ± 15.3 (0-52)	
Level I	0.5 ± 1.6 (0-7)		0.3 ± 1.2 (0-6)	

*See text.

†$p = 0.07$.

onset of delivery compared with placebo-treated women (Table III). Treatment effects differed with gestational age at time of rupture of membranes: (1) erythromycin-treated women enrolled between 28 to 32 weeks' gestation had significantly prolonged interval from rupture of membranes to delivery (Fig. 2; $p = 0.02$); (2) erythromycin treatment of maternal-infant pairs with preterm rupture of membranes <28 or 33 to 34 weeks' gestation was not associated with benefit. Maternal morbidity and microbial findings did not differ between treatment groups. Among subjects who received erythromycin, there were more neonatal deaths ascribed to immaturity. Intraventricular hemorrhage also occurred more frequently among newborns in the erythromycin group (five versus one). Three of these newborns in the erythromycin group were delivered at ≤26 weeks' gestation and were among the neonatal deaths. Overall, there was no improvement in perinatal survival among newborns of mothers who received erythromycin (Table IV). In the erythromycin-treated group, surviving newborns required less level II care ($p = 0.07$). Erythromycin treatment was well tolerated.

Prior studies evaluating antibiotic use among women with preterm PROM are difficult to compare because they used varying antibiotic regimens, study designs, or failed to control for potentially confounding factors such as gestational age, use of tocolytic agents, or corticosteroids.[9-13] Ohlsson[3] reviewed these and other studies and concluded that antibiotics should not be used outside of a randomized, controlled trial, such as this study.

Given methodologic differences, our findings most closely support those of Amon et al.[12] In an unblinded, randomized study, Amon[12] noted significantly prolonged time from study entry to delivery among women with preterm PROM treated with ampicillin compared with women who were not treated; 49% of ampicillin-treated women compared with 26% of untreated women continued their pregnancy for 1 week after enrollment ($p = 0.05$). In this study 52% of erythromycin-treated women and 26% of placebo-treated women continued their pregnancy at least 1 week after study entry ($p = 0.05$). As in our study, Amon[12] noted no reduction in maternal morbidity among antibiotic-treated women. The results of Amon's study,[12] as well as our overall finding of consistent trends for pregnancy prolongation (Table III), strengthen our finding of significant ($p = 0.02$) pregnancy prolongation among erythromycin-treated women who entered the study between 28 and 32 weeks' gestation.

In another trial of antibiotic use among women with PROM, Morales et al.[13] reported significant reduction in the occurrence of clinically recognized intraamniotic infection and neonatal sepsis only among women vaginally colonized with group B streptococcus who received ampicillin. However, the period from rupture of membranes to delivery did not differ between ampicillin-treated and untreated women. Interpretation of this study is complicated because ampicillin treatment differed with microbial findings. In the present study, effects of the study drug could not be correlated with the presence of specific bacteria. Lebherz et al.[10] reported no benefit in terms of reduced neonatal morbidity, perinatal mortality, or antenatal infection among women who received tetracycline after preterm

PROM. Lebherz[10] did note a significant reduction in postpartum maternal infectious morbidity (most importantly urinary tract infection) in women who received tetracycline. Infectious morbidity was low in our patients.

Erythromycin base was chosen as the study antimicrobial in this trial because it is safe for use in pregnancy, reasonably well tolerated, and active against various microorganisms implicated in PROM and chorioamnionitis. Erythromycin inhibits bacterial protein synthesis and may prevent release of bacterial factors such as proteases and phospholipases that may play roles in PROM and preterm birth.[8] Further, erythromycin treatment was associated with prolonged pregnancy and increased birth weight among women experiencing idiopathic preterm labor with intact membranes in two randomized, double-blind trials.[18, 19]

Erythromycin is neither well absorbed across the placenta nor fully active within vaginal fluid. Conversely, erythromycin achieves relatively high concentrations in maternal uterine tissues and fetal membranes. These characteristics may help explain the lack of adverse effects, including superinfection, as well as the gestational age–linked effects of erythromycin noted in this study. As noted, erythromycin treatment did not appear to prolong the latency period among women with PROM occurring at 33 to 34 weeks' gestation. Recovery of group B streptococcus, *C. trachomatis*, and *S. aureus* was more common among these women. The presence of such acknowledged pathogens and possibly untreated subclinical amniotic fluid infections, may have contributed to early onset of labor, as suggested by Newton.[20] Alternatively, reduced time between rupture of membranes and delivery closer to term (>32 weeks' gestation) may reflect relative maturation of physiologic processes associated with parturition.

Similarly, erythromycin was of no apparent benefit when rupture of membranes occurred <28 weeks' gestation. Hillier and Eschenbach[5] found that microbiologic or histologic evidence of chorioamnionitis is most commonly associated with preterm birth at such low gestational ages. In this study, the excess number of subjects with very low gestational ages randomized to the erythromycin treatment group may have obscured any beneficial effect from a limited (1 week) course of antibiotics. Alternatively, it is possible that effects of inflammatory processes already in place at study entry cannot be mitigated by orally administered erythromycin treatment alone. The significant effect of erythromycin administration at 28 to 32 weeks' gestation in this study suggests that erythromycin treatment effectively addresses factors that play important roles in PROM and subsequent preterm birth in this clinically important middle ground.

Future investigations for treatment of preterm PROM may include revision of empiric antibiotic treatment based on the individual patient's microbiologic findings, adjunctive antiinflammatory therapy, or more prolonged antibiotic treatment. The sudden increased slope of the survival analyses (Figs. 1 and 2) occurring approximately 3 days after cessation of erythromycin treatment suggests that continued therapy beyond 7 days may be beneficial.

This double-blind, randomized, placebo-control study supports suggestions that selected antimicrobial therapy may benefit some women with preterm PROM by prolonging their pregnancy and allowing for further fetal growth and development. Future controlled treatment trials as well as microbiologic and pathophysiologic studies are required to confirm these findings in other populations and to further define patient selection criteria, mechanisms of action, and possible untoward effects of such antimicrobial treatment. Knowledge gained from such studies may provide improved treatment and possibly preventive strategies for preterm PROM, especially before 28 weeks' gestation.

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Expression and amplification of the HER-2/*neu* (c-*erb*B-2) protooncogene in epithelial ovarian tumors and cell lines

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Amplification of the c-*erb*B-2 protooncogene has been associated with a poor prognosis in human breast and ovarian cancers. Our study was undertaken to examine whether amplification, rearrangement, or overexpression of c-*erb*B-2 and other protooncogenes was frequently observed in epithelial ovarian cancers. c-*erb*B-2 was expressed in 87% of 22 ovarian cancers analyzed, but expression was significantly increased in only one of the 22 tumor specimens. In this case elevated c-*erb*B-2 expression was associated with dramatic amplification of the gene. In another tumor a 3.8 kb *Eco*RI fragment was found, in addition to the usual 4.4 and 6.0 kb fragments; this is consistent with a possible gene rearrangement or a restriction fragment length polymorphism. To place these results in perspective, expression of several other protooncogenes has been examined in ovarian carcinomas. The c-*fos*, c-*myc*, n-*myc*, c-*fms*, and c-Ha-*ras* protooncogenes were expressed in different fractions of tumors, but expression of l-*myc*, c-*erb*B, c-*myb*, c-*sis*, and c-*mos* was not detectable. Aside from c-*erb*B-2, neither amplification nor rearrangement was observed among the other protooncogenes studied. Expression of c-*erb*B-2, c-*fms*, c-*myc*, n-*myc*, c-*fos*, and c-Ha-*ras* deserves further evaluation as a prognostic factor in ovarian cancer. (AM J OBSTET GYNECOL 1991;165:640-6.)

Key words: Protooncogenes in ovarian cancer, c-*erb* B-2, HER-2, *neu*

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Cellular protooncogenes play a central role in growth regulation, and activated oncogenes have been implicated in the malignant transformation of cells. Activation of oncogenes may occur by several different mechanisms. Point mutations in deoxyribonucleic acid (DNA) can produce altered protooncogene products associated with a malignant phenotype. Overexpression of unaltered protooncogenes also can be associated with transformation. Increased gene expression can result from amplification of gene sequences or from translocation of gene sequences to a site where they come under the influence of new enhancers or promotors.

Activation of several different oncogenes may be required to produce malignant transformation, and coordinate activation of certain oncogenes may be particularly important. Products of different protooncogenes include growth factors, receptors for growth factors, guanosine triphosphate binding proteins, and nuclear binding proteins. Whereas the growth regulatory network is apparently tightly controlled in normal cells, activation of one or more oncogenes may result in uncontrolled proliferation and acquisition of a malignant phenotype.

Activation of certain oncogenes have been described in a variety of human cancers, but relatively few studies have been performed in ovarian cancer.[1-6] This study was initiated to determine which protooncogenes are expressed, amplified, or rearranged in epithelial ovarian carcinomas.

Methods

Tissues. Portions of fresh malignant tissues were obtained at Duke University Medical Center and at the University of North Carolina between 1985 and 1987, in accordance with procedures approved by the appropriate institutional review boards for handling pathologic material. Tissues were snap frozen in liquid nitrogen and stored at − 70° C until analyzed. Overall, 33 epithelial ovarian cancers were studied, including 19 papillary serous carcinomas, 6 papillary adenocarcinomas, 3 poorly differentiated adenocarcinomas, 2 clear cell carcinomas, 1 endometrioid adenocarcinoma, 1 mucinous adenocarcinoma, and 1 mesonephric carcinoma.

Cell lines. The breast and ovarian carcinoma cell lines were obtained and maintained as described previously.[7]

DNA probes and probe labeling. Probes obtained from the American Type Culture Collection (Rockville, Md.) included c-*mos* (ATCC 41004), v-*myb* (ATCC 41012), v-*sis* (ATCC 41033), v-*erb*B-1 (ATCC 41019), and v-*fms* (ATCC 41017). Additional probes were obtained from the Duke Comprehensive Cancer Center DNA Probe Library (c-Ha-*ras*,[8] c-*fos*,[9] c-*myc*,[10] n-*myc*[11], and l-*myc*[12], Dr. Mary Carrington (γ-actin[13]), and Dr. Matthias Kraus (National Cancer Institute, Bethesda) (pMac 30, c-*erb*B-2, HER-2/*neu*[14]). The c-Ki-*ras* 2 probe was obtained from Oncor (Gaithersburg, Md.). Probes were radiolabeled with phosphorus 32–deoxycytidine triphosphate (3000 Ci/mmol; Amersham, Arlington Heights, Ill.) according to the Multiprime DNA labeling system (Amersham) according to the manufacturer's instructions.

Isolation of ribonucleic acid (RNA) and DNA. Frozen tissues were ground to a fine powder in liquid nitrogen. RNA was extracted with guanidium isothiocyanate and isolated on gradients of cesium chloride. DNA was recovered from the interphase of the step gradient used for RNA isolation. DNA was digested with ribonuclease I and pronase, extracted with phenol-chloroform, precipitated with ethanol, and dissolved in 10 mmol/L Tris hydrochloride buffer with 1 mmol/L ethylenediaminetetraacetate, pH 8. RNA and DNA preparations were checked for degradation by gel electrophoresis, and any degraded samples were discarded. All samples analyzed for oncogene expression or amplification were positive when hybridized with a γ-actin probe.

Northern blot analysis. Total cellular RNA (25 μg) was analyzed by electrophoresis in 1% agarose formaldehyde gel, then transferred to nitrocellulose paper (Schleicher and Schuell, Keene, N.H.) or a Gene Screen (New England Nuclear, Boston) filter in a Northern capillary transfer. For RNA dot blot hybridization, RNA (10 to 20 μg) was serially diluted and applied to nitrocellulose paper with a vacuum blotting apparatus (Schleicher and Schuell). Filters were baked in a vacuum oven at 80° C for 2 hours and prehybridized at 42° C for ≥15 minutes with 50% formamide, 10% dextran sulfate, and 1% sodium dodecyl sulfate in 1 mol/L sodium chloride. Labeled probe (0.5 to 1.0×10^6 cpm/ml) and salmon sperm DNA (0.8 mg/ml) were denatured and added to the filter. After hybridization overnight at 42° C, filters were washed twice in 2 × saline–sodium citrate buffer (SSC) with 1% sodium dodecyl sulfate at 25° C, then washed three times in 0.2 × SSC with 0.1% sodium dodecyl sulfate at 60° C, and subsequently exposed to x-ray film (Kodak X-Omat AR, Eastman Kodak, Rochester, N.Y.) at − 70° C with one intensifying screen.

Southern blot analysis. Genomic DNA (10 μg) was digested with appropriate restriction endonucleases (Bethesda Research Laboratories, Gaithersburg), electrophoresed in 0.7% or 1 % agarose gels, and blotted on nitrocellulose paper or a Gene Screen filter in a Southern capillary transfer. For DNA dot blot hybridization, DNA (10 to 20 μg) was serially diluted and applied to a Gene Screen filter with a vacuum blotting apparatus. Prehybridization and hybridization of filters were performed essentially as described above for Northern blot analysis. Filters were washed twice in 2 × SSC at 25° C, twice in 2 × SSC and 1% sodium dodecyl sulfate at 75° C, twice in 0.1 × SSC at 25° C, and autoradiographed as described above.

Monoclonal antibodies. TA-1, a monoclonal IgG1 antibody reactive with the extracytoplasmic domain of c-*erb*B-2, was provided by Dr. Sara McKenzie at Applied Biotechnology (Cambridge, Mass.).[15] Mopc-21

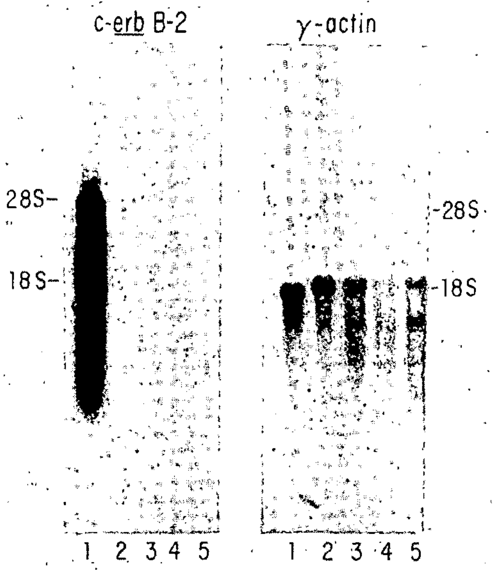

Fig. 1. Northern hybridization of ovarian carcinoma RNA. *Lanes 1 to 5* contain ICOV 46, ICOV 45, OV 111, OV 119, or ICOV 75 RNA probed with c-*erb*B-2 *(left panel)* or γ-actin DNA *(right panel)*.

Table I. Expression of protooncogenes in human epithelial ovarian carcinomas

Protooncogene	*No. positive per No. of tumors tested*	%
c-*fos*	9/9	100
c-*myc*	10/13	77
n-*myc*	4/14	28
l-*myc*	0/14	0
c-*fms*	8/14	57
c-Ha-*ras*	10/20	50
c-*erb*B-2	19/22	86
c-*myb*	0/14	0
c-*mos*	0/14	0
c-*sis*	0/14	0
c-*erb*B	0/14	0

monoclonal IgG1 antibody (American Type Culture Collection, Rockville, Md.) was used as a control for nonspecific binding of immunoglobulin. Antibodies were used at a concentration of 5 μg/ml for radioimmunoassays and 0.5 μg/ml for immunohistochemical analysis.

Live-cell radioimmunoassay. The live-cell radioimmunoassay was modified slightly from the method of Greiner et al.[7] Statistical significance of differences in counts was assessed with the Student *t* test.

Immunohistochemistry. Frozen sections (4 to 6 μm) were stained as described previously.[16]

Results

Expression of c-*erb* B-2 protooncogene in epithelial ovarian cancers. Nineteen of 22 RNA samples (87%) isolated from epithelial ovarian cancers expressed c-*erb*B-2 by Northern blot analysis (Table I). The expected 4.4 kb c-*erb*B-2 transcript migrates just below the 28S ribosomal RNA band (Fig. 1). One tumor (ICOV 46) overexpressed c-*erb*B-2 RNA, although its expression of γ-actin RNA was comparable to that of other tumors (Fig. 1). Dot blot analysis of the same five tumor RNA samples indicated a 64-fold overexpression of c-*erb*B-2 RNA in the ICOV 46 tumor relative to the other tumors (Fig. 2). Five ovarian cancer cell lines derived from ascites fluid of patients with epithelial ovarian cancer and a B-lymphoblastoid cell line from one of the five patients were evaluated for expression of c-*erb*B-2 RNA by dot blot analysis. RNA isolated from the SKBr-3 breast cancer cell line, which overexpressed c-*erb*B-2 RNA, was included as a positive control. Among the ovarian cancer cell lines, OVCA 429 ex-

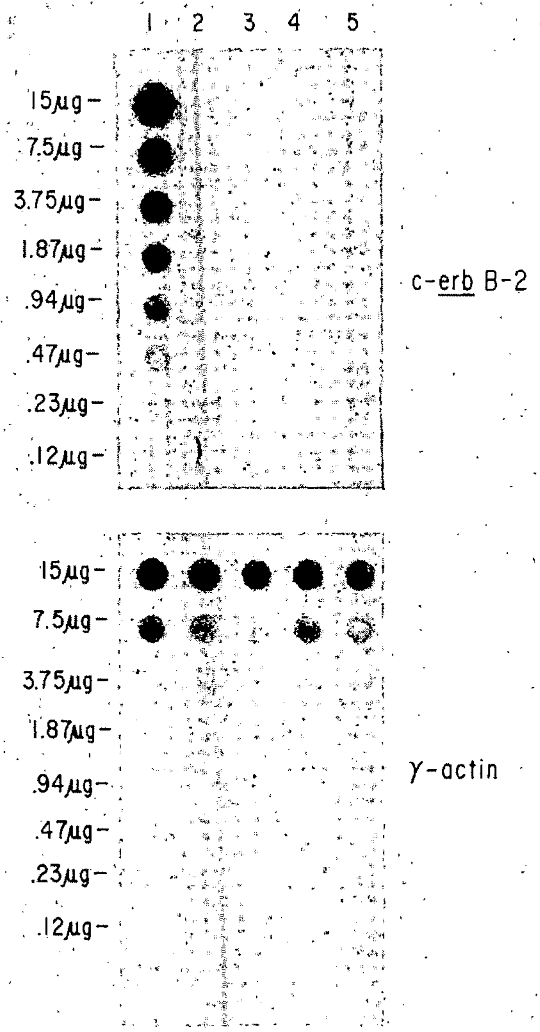

Fig. 2. Dot blot hybridization of ovarian carcinoma RNA. *Lanes 1 to 5* contain ICOV 46, ICOV 45, OV 111, OV 119, or ICOV 75 RNA probed with c-*erb*B-2 *(upper panel)* or γ-actin DNA *(lower panel)*.

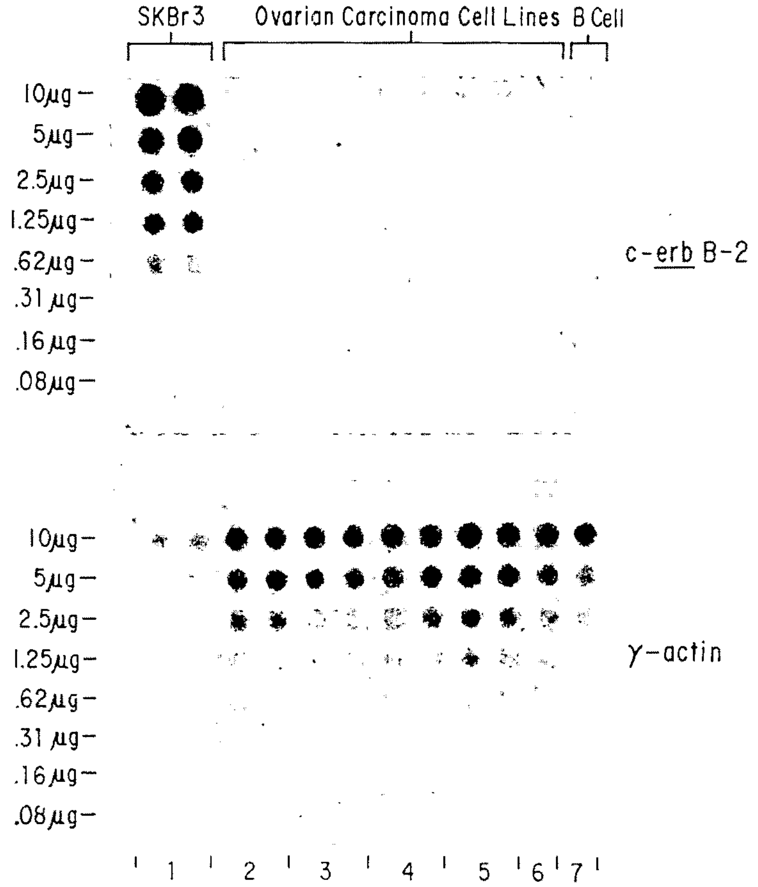

Fig. 3. Dot blot hybridization of breast and ovarian carcinoma cell line RNA. *Lanes 1 to 5* contain duplicate samples of SKBr-3, OVCA 433, OVCA 432, OVCA 420, or OVCA 429 RNA. *Lanes 6 and 7* contain single samples of ovarian cancer cell line DOV 13 and autologous B-lymphoblastoid cell line RNA, respectively. *Upper panel* was probed with c-*erb*B-2 DNA and *lower panel* with γ-actin DNA.

pressed the most c-*erb*B-2 RNA; however, the level of expression was 32-fold less than the level in SKBr 3 cells (Fig. 3). B-lymphoblastoid cell lines did not express detectable levels of c-*erb*B-2 RNA. RNA from each of the cell lines expressed comparable levels of reactivity with a γ-actin probe (Fig. 3).

RNA from epithelial ovarian cancers was also evaluated for expression of other protooncogenes by Northern blot analysis. In addition to c-*erb*B-2, the c-*fos*, c-*myc*, c-*fms*, and C-Ha-*ras* protooncogenes were expressed in a majority of the tumors tested. Expression of n-*myc* was found in one third of tumors. No hybridization was detected with probes for l-*myc*, v-*myb*, c-*mos*, v-*sis*, and c-*erb*B, although very low levels of these transcripts would not be discerned by Northern blot analysis of total cellular RNA (Table I).

Amplification of c-erbB-2 and other protooncogenes in epithelial ovarian cancers. DNA samples were isolated fom a total of 26 patients with ovarian cancer and evaluated for amplification of the c-*erb*B-2 gene by Southern blot analysis. In 13 cases DNA was obtained from samples that have been analyzed for expression of c-*erb*B-2 RNA. Amplification of c-*erb*B-2 was observed in DNA from one tumor (ICOV 46, Fig. 4). Dot blot analysis (Fig. 5) indicated a 64-fold amplification in the same tumor (ICOV 46) that had overexpressed c-*erb*B-2 on Northern transfers (Figs. 1 and 2). No amplification of c-*erb*B-2 DNA was detected in the ovarian cancer cell lines (data not shown). In each of 22 DNA specimens examined, neither amplification nor rearrangement was observed with any of the probes previously used for analysis of gene expression.

Restriction fragment length polymorphism of the c-erbB-2 protooncogene in epithelial ovarian cancer. The c-*erb*B-2 probe pMAC 30 generally hybridizes with two restriction fragments, 6.6 and 4.4 kb in length, in an *Eco*RI digest of DNA (Fig. 4). Hybridization with an additional 3.8 kb restriction fragment was observed with one tumor DNA (ICOV 21, Fig. 6), indicating a possible rearrangement of the c-*erb*B-2 gene. No c-*erb*B-2 polymorphism was evident when ICOV 21 DNA was digested with *Xba* I, or *Hin*dIII, rather than

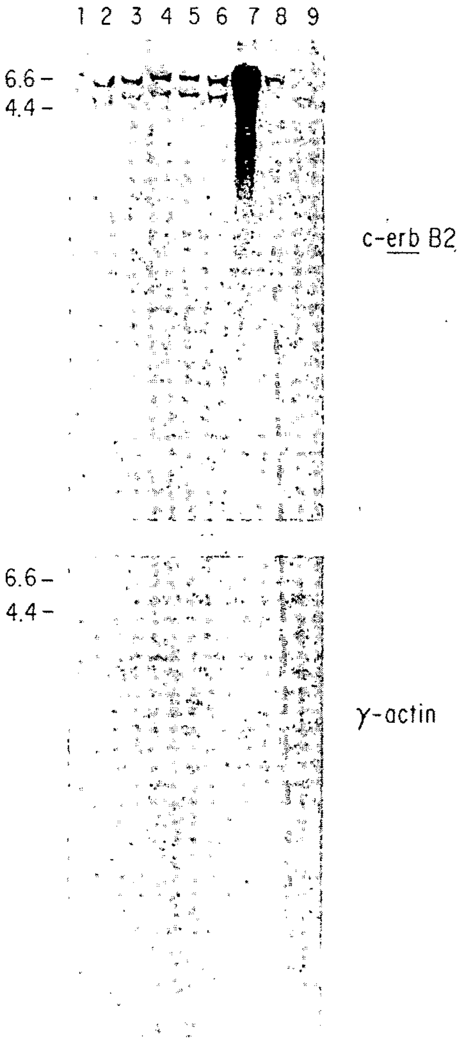

Fig. 4. Southern hybridization of ovarian carcinoma DNA. *Lanes 1 to 9* contain ICOV 10, ICOV 21, ICOV 31, ICOV 36, ICOV 43, ICOV 44, ICOV 46, ICOV 57, or ICOV 67 DNA probed with c-*erb*B-2 *(upper panel)* or γ-actin DNA *(lower panel)*.

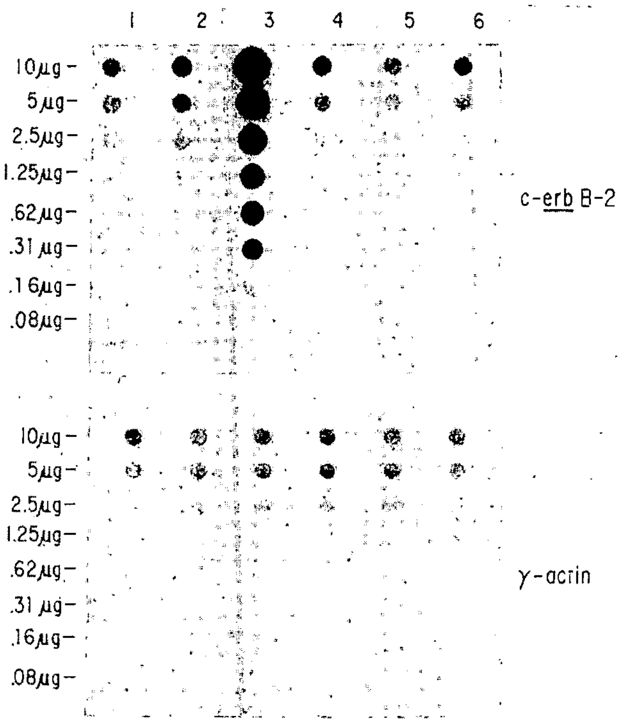

Fig. 5. Dot blot hybridization of ovarian carcinoma DNA. Lanes 1 to 6 contain ICOV 36, ICOV 43, ICOV 44, ICOV 46, ICOV 57, or ICOV 67 DNA probed with c-*erb*B-2 DNA *(upper panel)* or γ-actin DNA *(lower panel)*.

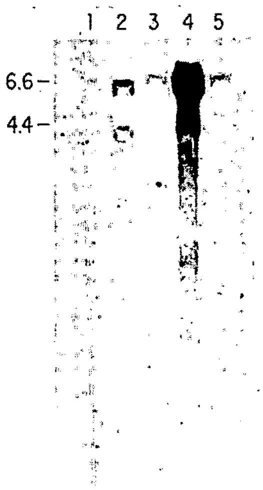

Fig. 6. Restriction fragment length polymorphism c-*erb* B-2 in ovarian carcinoma DNA. Southern hybridization of ovarian cancer DNA from ICOV 15, ICOV 21, ICOV 36, ICOV 46, and ICOV 57 in *lanes 1 to 5* probed with c-*erb*B-2.

*Eco*RI (data not shown). In another tumor DNA sample (ICOV 57), hybridization was observed only with the 6.6 kb restriction fragment, although both fragments were observed in normal DNA from the same individual (data not shown).

Detection of the c-erbB-2 protein in epithelial ovarian cancers. In a separate study[16] staining of frozen sections of normal and cancerous ovarian epithelium with the TA-1 anti-c-*erb*B-2 monoclonal antibody by the avidin-biotin immunoperoxidase method had been scored as no staining (0), light staining (1+), moderate staining (2+), or heavy staining (3+). Frozen sections of 13 tumors that had also been evaluated for expression or amplification of c-*erb*B-2 RNA or DNA were available for immunoperoxidase staining. All of the ovarian cancer tissues expressed c-*erb*B-2 protein. Two of 13 ovarian cancer tissues had light staining, two had moderate staining, and 9 of 13 had heavy staining, including ICOV 46.

Ovarian cancer cell lines were evaluated for surface expression of c-*erb*B-2 in a live-cell radioimmunoassay with the TA-1 monoclonal antibody. SKBr-3 and MCF-7 breast cancer cell lines were included as controls

Table II. Expression of c-*erb*B-2 protein in breast and epithelial ovarian cancer cell lines

		Antibody binding† (counts/min, mean ± SD)	
Cell line	*Mopc-21**	*TA-1**	*TA-1/Mopc-21*
SKBr-3	309 ± 46	2829 ± 121†	9.2
MCF7	292 ± 37	438 ± 50‡	1.5
OVCA 420	318 ± 65	593 ± 52‡	1.9
OVCA 429	252 ± 56	836 ± 164‡	3.3
OVCA 432	264 ± 24	310 ± 56	1.2
OVCA 433	336 ± 7	560 ± 83‡	1.7
Human fibroblast	281 ± 5	246 ± 46	0.9

*Purified antibody at five per milliliter.

†$p < 0.01$, versus Mopc-21 control, Student *t* test.

‡$p < 0.05$, versus Mopc-21 control, Student *t* test.

that overexpressed c-*erb*B-2 (SKBr-3) or expressed normal levels of the protein (MCF-7). SKBr-3 cells bound nine fold more TA-1 monoclonal antibody than control Mopc-21 antibody, whereas MCF-7 cells bound 1.5-fold more TA-1 than Mopc-21 (Table II). OVCA 429 had the highest surface c-*erb*B-2 expression of the ovarian cancer cell lines (TA-1/Mopc-21 ratio, 3.3). Its RNA also had the most hybridization among the ovarian cancer cell lines with the c-*erb*B-2 probe by dot blot analysis (Fig. 3). Two additional ovarian cancer cell lines (OVCA 420 and OVCA 433) had surface c-*erb*B-2 expression comparable to that of MCF-7. One ovarian cancer cell line (OVCA 432) and a human fibroblast cell line did not bind signficant amounts of TA-1 antibody (Table II).

Comment

Overexpression of the c-*erb*B-2 (HER-2/*neu*) protooncogene has been associated with a poor prognosis in both breast and ovarian cancer.[5, 16-19] The reported incidence of c-*erb*B-2 gene amplification has ranged from 1 in 26 (4%) in our study to 31 in 120 (26%) in the study by Slamon et al.[5] Three additional reports described intermediate frequencies of c-*erb*B-2 amplification in ovarian cancers: 1 in 11 (9%),[1] 3 in 15 (20%),[20] and 1 in 5 (20%). Overall, in studies to date amplification of c-*erb*B-2 has been observed in 21% of 177 tumors evaluated. Some of the variation in observed frequency may be due to the small sample size in most of the studies. Another possibility is that the proportion of stromal cells might vary from tumor to tumor. Amplified c-*erb*B-2 sequences in epithelial ovarian cancer cells might be diluted by DNA of stromal cells that contain only single copies of the gene. Degradation of DNA or RNA also could affect the level of hybridization in Northern and Southern transfers, but the relatively low incidence of amplification and overexpression in our study was not due to this factor, since both DNA and RNA were tested for integrity before evaluation. All samples analyzed for oncogene amplification or expression contained intact nucleic acids, since they hybridized with a γ-actin probe.

Slamon et al.[5] found a complete correlation between gene amplification and overexpression of c-*erb*B-2 RNA or protein in breast and ovarian cancers. All of the tumors with amplified genes also were overexpressed. In addition, there was about a 10% incidence of cancers that had single-copy levels of the c-*erb*B-2 gene but also had evidence of overexpression by Northern blot analysis or immunoperoxidase staining. In our study c-*erb*B-2 DNA and RNA levels in the tumors were comparable, except for one tumor with marked amplification and overexpression of the gene. Immunoperoxidase staining of ovarian cancers, however, showed heavy staining for c-*erb*B-2 protein in the majority (9/13) of these tumors. In a larger study of 73 patients with ovarian cancer 32% of tumors exhibited increased levels of c-*erb*B-2 protein by immunoperoxidase staining.[16] Observation of increased levels of gene product in the absence of apparent amplification or overexpression of messenger RNA suggests that post-transcriptional regulation deserves further evaluation.

Only one restriction fragment length polymorphism, an additional 3.4 kb *Eco*RI fragment in one breast cancer, previously has been reported for c-*erb*B-2. Using a different probe we observed an extra 3.8 kb *Eco*RI fragment in one ovarian cancer, indicating either the creation of an additional polymorphic *Eco*RI site in a structurally normal gene or a possible rearrangement in or near the c-*erb*B-2 gene. In another patient the expected 4.4 kb *Eco*RI fragment was missing, indicating a possible deletion or point mutation in or near the c-*erb*B-2 gene.

A high frequency of expression of c-*myc* and c-*fos* was not unexpected because the expression of these protooncogenes is associated with proliferating cells. The $p62^{myc}$ product has been found to be expressed at higher levels in malignant ovarian cancer than in benign neoplasms or normal ovary. Although the c-Ha-

ras oncogene was expressed in half of the ovarian cancers tested, no overexpression or amplification was detected. Amplification of c-Ha-*ras* has been reported in 1 of 61 tumors (2%) studied by different investigators. Mutation of c-Ki-*ras* has been reported in a single case of ovarian cancer, and amplification was observed in 9 of 61 tumors (15%) studied to date.[2, 3]

The high frequency of expression of the *fms* oncogene in ovarian cancers is in agreement with data recently reported by Kacinski et al.[6] Macrophages also are known to express *fms*. The *fms* oncogene product is homologous to the receptor for macrophage colony-stimulating factor, a growth factor that stimulates monocytopoiesis and is a potent chemoattractant for macrophages. Of interest is that most ovarian cancer cell lines express macrophage colony-stimulating factor RNA and secrete macrophage colony-stimulating factor into culture medium. Thus the presence of a growth factor and its receptor in the same cells could provide autocrine stimulation of ovarian tumor growth or autocrine modification of cellular function. To date we have been unable to demonstrate stimulation of ovarian cancer cell growth with recombinant macrophage colony-stimulating factor. Alternatively, the paracrine growth regulatory mechanism might result from ovarian cancer cells that produced macrophage colony-stimulating factor, recruiting macrophages to the peritoneal cavity and tumor sites. Macrophage-derived growth factors could, in turn, stimulate ovarian cancer cell growth, as has been reported in murine sarcomas infiltrated with macrophages. We have observed that several macrophage-derived factors, including interleukin-1, tumor necrosis factor, interleukin-6, and an apparently novel factor, stimulate the growth of ovarian cancer cell lines. Identification of the components of this potential paracrine growth stimulatory loop in ovarian cancer may provide information useful in devising strategies for therapeutic intervention in ovarian cancer.

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Simultaneous measurements of prostacyclin and thromboxane metabolites during the menstrual cycle

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The balance between prostacyclin and thromboxane is known to be important for reproductive and cardiovascular health but has been difficult to quantify. Whereas their stable urinary metabolites, 6-keto-prostaglandin $F_{1\alpha}$ and thromboxane B_2, may reflect in part systemic changes, it has been suggested that 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ and 11-dehydro-thromboxane B_2 more adequately reflect systemic production. Therefore we report for the first time the simultaneous measurements of 6-keto-prostaglandin $F_{1\alpha}$, thromboxane B_2, 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, and 11-dehydro-thromboxane B_2 during the menstrual cycle. Timed urine collections were obtained from 18 ovulatory women, aged 22 to 40, during the midfollicular and midluteal phases. Serum estradiol, progesterone, urinary pregnanediol glucuronide, estrone glucuronide, and creatinine also were measured. Prostanoid extraction from urine by C_{18} and silica Bond Elut columns were then separated by high-performance liquid chromatography before radioimmunoassay. Concentrations of all urinary prostanoids were not significantly different in the follicular phase when compared with the luteal phase. A positive correlation of borderline significance was noted between luteal thromboxane B_2 and pregnanediol glucuronide ($r = 0.70$) and between luteal estrone glucuronide and 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ ($r = 0.68$). A significant correlation was found between follicular estrone glucuronide and the 6-keto-prostaglandin $F_{1\alpha}$/11-dehydro-thromboxane B_2 ratio ($r = 0.83$, $p < 0.04$). These novel normative data suggest an influence of sex steroids on prostacyclin and thromboxane metabolism. (AM J OBSTET GYNECOL 1991;165:647-51.)

Key words: Prostacylin, thromboxane, menstrual cycle, hormone mediated

Prostaglandins have been identified in every human reproductive tissue. Numerous previous studies with blood, endometrial tissue, menstrual fluid, or urine have measured prostaglandin E (PGE) and prostaglandin F (PGF) during the menstrual cycle.[1, 2] To date, however, only a small number of studies have assessed prostacyclin and thromboxane balance by the measurements of their respective stable metabolites, 6-keto-$PGF_{1\alpha}$ and thromboxane B_2.[3-5] Prostacyclin/thromboxane balance is considered to be important for normal vascular physiology and may be aberrant in abnormal states, ranging from pregnancy-induced hypertension[6] to atherosclerosis.[7]

Prostacyclin and thromboxane have been difficult to assess because these prostanoids are rapidly cleared from the blood due to their short half-lives. In addition, blood sampling with associated blood vessel trauma may result in artifactual increases mediated by platelet release and aggregation.[8-10] The measurement of urinary metabolites, which avoids the sampling problems associated with other invasive methods, is therefore considered to be the preferred method for assessing levels of prostacyclin and thromboxane.

Urinary 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 are likely to reflect renal vascular tone, but it is uncertain whether they reflect systemic production.[11, 12] Their metabolites, 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ and 11-dehydro-thromboxane B_2, are thought to reflect systemic production.[13, 14] Therefore we have developed assays for these metabolites and report for the first time the simultaneous measurements of 6-keto-$PGF_{1\alpha}$, 2,3-dinor-$PGF_{1\alpha}$, thromboxane B_2 and 11-dehydro-thromboxane B_2 during the menstrual cycle and how these metabolites correlate with estrogen and progesterone excretion.

Material and methods

Subjects and protocol. Eighteen healthy premenopausal women, aged 22 to 40 years (mean, 33 years),

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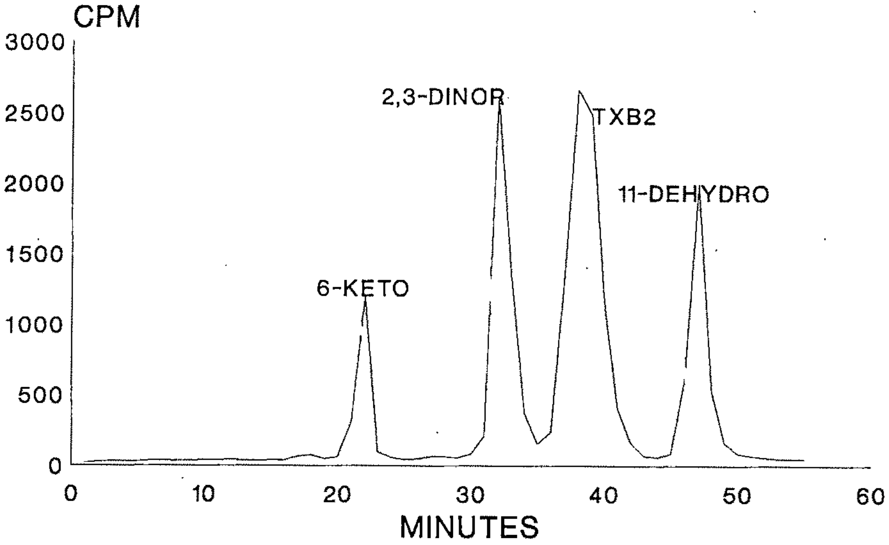

Fig. 1. Sample of high-performance liquid chromatography chromatogram for simultaneous separation of four prostaglandin metabolites, 6-keto-PGF$_{1\alpha}$, 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$, thromboxane B$_2$, and 11-dehydro-thromboxane B$_2$.

were recruited for the study. No subject had a history of significant illness (e.g., diabetes mellitus, hypertension, or cardiovascular, pulmonary, peripheral vascular, or renal disease). All women were within 20% of ideal body weight (mean, 136 pounds) and were nonsmokers. Subjects menstruated regularly with cycle lengths between 24 and 30 days (mean, 28 days). The cycle phase and ovulatory status were determined with the history of the last menstrual period and confirmed by a ratio of urinary pregnanediol glucuronide to creatinine (>2 $\mu g \cdot mg^{-1}$), as previously described.[15]

Subjects were asked not to consume aspirin, nonsteroidal antiinflammatory agents, or alcohol at least 2 weeks before or during the study period. In addition, no subjects had received any exogenous sex steroids within 60 days of the study. The women were studied in the midfollicular phase on days 5 to 9 ($n = 10$) or during the midluteal phase on days 19 to 24 ($n = 8$). Timed overnight urine samples were obtained and stored at −20° C until analyzed for the prostanoids. Simultaneous measurements of serum estradiol, progesterone, urinary pregnanediol glucuronide, estrone glucuronide, and creatinine also were obtained.

Assays

Prostanoids. Urinary 6-keto-PGF$_{1\alpha}$, 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$, thromboxane B$_2$, and 11-dehydro-thromboxane B$_2$ were measured by high-performance liquid chromatography radioimmunoassay (RIA) as follows: First, a 20 ml aliquot was obtained from an overnight urine sample, centrifuged to remove any sediment, and then acidified with formic acid to pH 3.5. Next, these internal standards were added to monitor procedural losses: approximately 4000 disintegrations/min of ^{3}H-6-keto-PGF$_{1\alpha}$ (Amersham Corp., Arlington Heights, Ill.; specific activity, 157 Ci/mmol); ^{3}H-2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$ (Amersham; specific activity, 157 Ci/mmol; prepared by Brass et al.[16] and purified in our laboratory by high-performance liquid chromatography); ^{3}H-thromboxane B$_2$ (NEN Research Products, Boston; specific activity, 180 Ci/mmol); and ^{3}H-11-dehydro-thromboxane B$_2$ (Amersham; specific activity, 179 Ci/mmol), each in 0.1 ml of assay buffer. Prostanoids were then extracted from the urine with a sequential elution on a Bond Elut C$_{18}$ (3 ml) and silica (3 ml) column (Analytichem International, Harbor City, Calif.). Samples were then dried down with nitrogen at 40° C, and the residues were dissolved in 60 µl methanol.

Separation of the reconstituted sample was accomplished through chromatography, with a high-performance liquid chromatography system consisting of two model 501 pumps, a model 680 automated gradient controller, a model 481 variable wavelength detector, a model 712 Waters Intelligent Sample Processor, and a model 745 data module (Waters Associates, Inc., Milford, Mass.), and quantification of the separated fractions was achieved by RIA. Specifically, separation of 6-keto-PGF$_{1\alpha}$ (t_R [retention time] = 19.5 to 24.5 minutes), 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$ (t_R = 30 to 34 minutes), thromboxane B$_2$ (t_R = 35 to 40 minutes), and

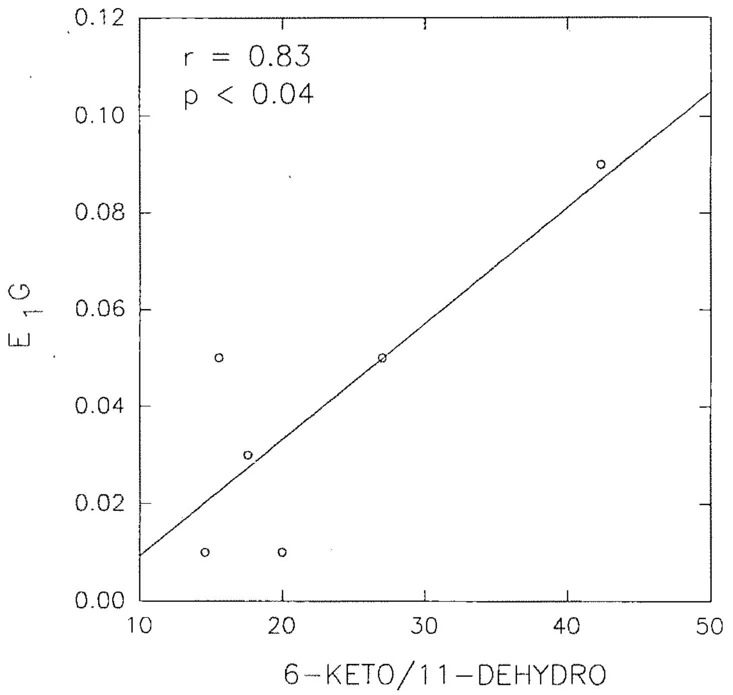

Fig. 2. Correlation of follicular urinary estrone glucuronide *(E_1G)* in micrograms per gram creatinine with 6-keto-$PGF_{1\alpha}$/11-dehydro-thromboxane B_2 ratio in nanograms per gram creatinine.

11-dehydro-thromboxane B_2 (t_R = 45 to 49 minutes) (Fig. 1) was performed through reverse-phase high-performance liquid chromatography with a programmed gradient prepared from water/trifluoroacetic acid (100:0.05, solvent A) and acetonitrile/trifluoroacetic acid (100:0.05, solvent B). Eluates were then evaporated under a vacuum by means of a vortex evaporator (Buchler Instruments, Fort Lee, N.J.). Prostanoid residues were then reconstituted in 0.7 ml buffer, and 0.2 ml aliquots in duplicate were taken for RIA; an additional 0.2 ml aliquot was taken for determination of procedural losses. The 6-keto-$PGF_{1\alpha}$ RIA uses ^3H-6-keto-$PGF_{1\alpha}$ (20,000 disintegrations/min) in conjunction with anti-6-keto-$PGF_{1\alpha}$ bovine serum albumin (final dilution, 1:100,000); the thromboxane B_2 RIA is carried out with anti-thromboxane B_2 bovine serum albumin (final dilution, 1:60,000) and approximately 20,000 disintegrations/min of ^3H-thromboxane B_2. The 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ RIA uses the anti-6-keto-$PGF_{1\alpha}$ serum, which cross reacts with 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$ (10,000 disintegrations/min) in conjunction with ^3H-2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$. Because 11-dehydro-thromboxane B_2 exists in cyclic and acyclic forms in a pH-dependent equilibrium, a specific buffer (0.2 mol/L Tris-phosphate hydrochloride, pH 9.2) was used to maximize the sensitivity and specificity of the RIA. In each RIA after a 16- to 18-hour incubation at 4° C, the free and antibody-bound prostanoids were separated by adding 0.6 ml of a dextran-coated charcoal (Norit A) suspension (0.5% [wt/vol]) and 0.05% (wt/vol) dextran. The antibody-bound fraction was then counted.

The intraassay coefficients of variation for 6-keto-$PGF_{1\alpha}$, 2,3-dinor-6-keto-prostaglandin $F_{1\alpha}$, thromboxane B_2, and 11-dehydro-thromboxane B_2 were 8.9, 10.0, 6.2, and 18.7, respectively, whereas the interassay coefficients of variation were 15.3, 6.6, 17.3, and 15.7, respectively.

Hormones. Estradiol, progesterone, pregnanediol glucuronide, and estrone glucuronide were all measured with established RIAs.[17]

Creatinine. Urinary creatinine was measured with the modified Jaffe reaction.

Statistics. Statistical analyses used two-sample *t* testing and regression analysis by the method of least squares. All results are reported as mean ± SE and calculated in nanograms per gram creatinine in the case of prostanoids.

Results

Prostanoid concentrations. Follicular and luteal phase estradiol levels were 66 ± 10 and 217 ± 29 pg/ml, respectively, whereas the respective follicular

Table I. Prostaglandin and thromboxane metabolites and their ratios in follicular and luteal phases*, †

	No.	6-Keto-PGF$_{1\alpha}$ (ng/gm)	No.	2,3-Dinor-6-keto-PGF$_{1\alpha}$ (ng/gm)	No.	11-Dehydro-thromboxane B$_2$ (ng/gm)	No.	Thromboxane B$_2$ (ng/gm)
Follicular	6	20.3 ± 6.9	10	173.5 ± 32.4	10	876.9 ± 123.1	10	13.8 ± 3.0
Luteal	7	31.8 ± 7.0	7	261.8 ± 59.2	7	678.6 ± 101.8	7	15.5 ± 4.3

*Mean ± SE.

†Values are in nanograms per gram creatinine.

and luteal progesterone levels were 0.17 ± 0.05 and 15 ± 3 ng/ml.

The concentrations of 6-keto-PGF$_{1\alpha}$, 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$, thromboxane B$_2$, and 11-dehydro-thromboxane B$_2$ and their ratios during the follicular and luteal phases are summarized in Table I. Concentrations of all urinary prostanoids were not significantly different in the follicular phase when compared with the luteal phase. All meaningful ratios between prostacyclin and thromboxane metabolites during the two phases of the cycle were calculated (data not depicted). No significant changes were noted.

When urinary prostanoids are compared with urinary pregnanediol glucuronide, a positive correlation of borderline significance was noted between luteal thromboxane B$_2$ and pregnanediol glucuronide ($r = 0.70$, $p = 0.07$) and between luteal estrone glucuronide and 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$ ($r = 0.68, p < 0.09$). In the follicular phase a significant positive correlation was evident between estrone glucuronide and the 6-keto-PGF$_{1\alpha}$/11-dehydro-thromboxane B$_2$ ratio ($r = 0.83, p < 0.04$) (Fig. 2). However, correlations between follicular and luteal 6-keto-PGF$_{1\alpha}$ and its stable metabolite, 2,3-dinor, or thromboxane B$_2$ and its metabolite, 11-dehydro-thromboxane B$_2$, failed to show significance ($r = 0.33, p = 0.5$; $r = 0.05, p = 0.9$; $r = 0.37$, $p = 0.3$; $r = 0.06$, $p = 0.9$, respectively).

Comment

Previous studies assessing prostaglandins during the menstrual cycle have focused on PGF$_{2\alpha}$ and PGE$_2$. We have presented for the first time a novel approach to the measurement of the stable metabolites of prostaglandins prostacyclin and thromboxane. These stable metabolites, 6-keto-PGF$_{1\alpha}$ and thromboxane B$_2$, and the 2,3-dinor and 11-dehydro metabolites have been simultaneously measured during the menstrual cycle.

In vivo and in vitro studies point to the role of estrogen in increasing prostacyclin biosynthesis and that progesterone decreases this apparently estrogen-mediated effect.[18-20] Recent evidence suggesting that prostacyclin production is significantly decreased in uterine arteries from postmenopausal women[21] further enhances this notion. Ylikorkala et al.[3] have shown an increase in 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$, using spot urine samples, from subjects given a regimen of oral contraceptive tablets containing desogestrel and ethinyl estradiol. No change, however, was noted in thromboxane B$_2$.

Prostacyclin, formed by the vascular endothelium, a potent vasodilator and the most powerful inhibitor of platelet aggregation, has had its uterine biosynthesis localized primarily to the myometrium.[22] It is reasonable to suggest that estrogen may increase prostacyclin and that this may influence blood flow to the myometrium. The precise function of thromboxane in the uterus, however, is unclear. Thromboxane, derived from platelets, is a proaggregator and powerful vasoconstrictor that can lead to the development of ischemia and uterine pain. In vitro studies indicate that it can stimulate the myometrium and all three muscle layers of the uterotubal junction to contract, thus increasing overall uterine tonus.[23]

The values we obtained for the various prostaglandin measurements are similar to those noted by other investigators.[24] The positive correlations of urinary estrone glucuronide to luteal 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$ and to the follicular ratio of 6-keto-PGF$_{1\alpha}$/11-dehydro-thromboxane B$_2$ are in keeping with the physiologic concept of hormone-mediated changes in prostaglandins. Also in keeping with these tenets is the positive correlation between luteal phase thromboxane and pregnanedial glucuronide. Liggins et al.[4] showed by using perifusion of human endometrial tissue during the menstrual cycle that the 6-keto-PGF$_{1\alpha}$/thromboxane B$_2$ ratio, which factored 6-keto-PGF$_{1\alpha}$ in the proliferative phase, favored thromboxane B$_2$ in the premenstrual phase. A possible role of thromboxane in limiting menstrual blood loss and preventing retrograde menstrual bleeding is also suggested by data that thromboxane may contract the fallopian tube and that platelets are present in menstrual blood.[23]

Although our study did not show any differences in 6-keto-PGF$_{1\alpha}$, 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$, thromboxane B$_2$, or 11-dehydro-thromboxane B$_2$ during the menstrual cycle, there was the suggestion, as noted, that changes in prostanoids occurred in association with hormonal fluctuations. Although previous work has suggested that 2,3-dinor-6-keto-prostaglandin F$_{1\alpha}$ and 11-dehydro-thromboxane B$_2$ urinary me-

No.	6-Keto-PGF$_{1\alpha}$/thromboxane B$_2$ (ng/gm)	No.	6-Keto-PGF$_{1\alpha}$/11-dehydro-thromboxane B$_2$ (ng/gm)	No.	2,3-Dinor-6-keto-PGF$_{1\alpha}$/11-dehydro-thromboxane B$_2$ (ng/gm)	No.	2,3-Dinor-6-keto-PGF$_{1\alpha}$/thromboxane B$_2$ (ng/gm)
6	1.7 ± .41	6	.04 ± 0.01	10	0.27 ± 0.09	10	25.6 ± 8.4
7	2.4 ± .53	7	0.04 ± 0.01	7	0.34 ± 0.04	4	23.2 ± 8.9

tabolites may be more reflective of systemic production than either 6-keto-PGF$_{1\alpha}$ or thromboxane B$_2$, the metabolites of prostacyclin and thromboxane were similar during the menstrual cycle. This observation need not be contradictory in that we studied normal ovulatory women who did not have systemic disease. Further, the hormonal changes during the menstrual cycle are rapid and transient. More prolonged hormonal changes may be required to modify prostanoid secretion in the directions suggested.

This report therefore provides novel normative data on prostacyclin and thromboxane during the normal menstrual cycle, including the method for the simultaneous measurements of their stable metabolites. We are hopeful that this information will help provide a reference point for more investigation in this area.

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Recurrent mild abruptio placentae occurring immediately after repeated electroconvulsive therapy in pregnancy

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We present a case in which electroconvulsive therapy was performed repeatedly in pregnancy because of severe depression with psychotic features and failure of chemical treatment. Each electroconvulsive treatment was immediately followed by uterine contractions and active uterine bleeding, possibly representing recurrent abruptio placentae occurring in association with the treatment. Transient acute episodes of maternal hypertension between 180/90 and 190/100 mm Hg, documented within minutes after application of each electroconvulsive treatment, might explain the abruptio placentae manifested by active uterine bleeding and uterine hyperstimulation. (AM J OBSTET GYNECOL 1991;165:652-3.)

Key words: Pregnancy, electroconvulsive therapy, abruptio placentae

The relative safety of electroconvulsive therapy in the treatment of certain cases of depression, mania, or psychosis in pregnancy has been reported in both obstetric and psychiatric literature.[1, 2] To the best of our knowledge, adverse outcomes with the application of this therapy in pregnancy have not been reported. We present a case in which repeated electroconvulsive therapy was performed in pregnancy. After each session of electroconvulsive therapy, mild abruptio placentae was suspected. We discuss the possible underlying mechanisms.

Case report

A 35-year-old Oriental woman, gravida 2, para 0, with one previous elective termination of pregnancy, was monitored during her second gestation. Her medical history was unremarkable. Gestational dating was based on last menstrual period and 9-week ultrasonography. The patient declined a genetic amniocentesis. At 19 weeks' gestation the patient began experiencing insomnia, anxiety attacks, and occasional suicidal thoughts. Treatment with oral diazepam, 5 mg three times a day, was initiated. Initially the patient responded. Ultrasonographic evaluation at 21 weeks revealed normal fetal anatomy and a low-lying posterior placenta. The patient's psychiatric condition deteriorated at 25 weeks' gestation with signs of worsening depression. After a suicide attempt at 26 weeks, she was hospitalized at an inpatient psychiatric hospital with a diagnosis of severe depression with psychotic features. Her condition remained unchanged, and antipsychotic therapy consisting of oral haloperidol (Haldol), 10 mg twice a day, was added. At 30 weeks, because of the intensity of delusions, severe anorexia, continued suicidal ideations, and advancing pregnancy, a decision was made to move directly to electroconvulsive therapy with the patient under general anesthesia and with continuous cardiotocographic monitoring. Before the electroconvulsive therapy, intravenous fluids were administered to avoid hypovolemia, and the blood pressure was 106/68 mm Hg. She was given oxygen by mask and intubated after intravenous thiopental sodium, 125 mg, and succinylcholine, 50 mg. Electroconvulsive therapy was performed with standard bilateral temporal electrode placement and a 30% energy setting (pulsed bidirectional square-wave stimulus with a fixed pulse width of 1 msec and a frequency of 70 Hz; Thymatron Somatics, Inc., Lake Bluff, Ill.), resulting in a seizure lasting 50 seconds. Immediately after the seizure, transient hypertension with pressures of 180/90 mm Hg was noted in conjunction with visible, palpable regular uterine contractions every 2 to 3 minutes. The fetal heart rate revealed reduced variability that was attributed to the intravenous thiopental sodium and was otherwise normal (Fig. 1). Uterine hyperstimulation (Fig. 1) was noted in conjunction with mild, bright red uterine bleeding. Gradually the bleeding and uterine contractions subsided. This regimen was performed on a weekly basis, with the same clinical picture after each session of electroconvulsive therapy. The uterine hyperstimulation noted after the electroconvulsive therapy at 31 weeks necessitated subcutaneous terbutaline, 2.5 mg. At 32 weeks' gestation Doppler velocimetric studies of the uterine and umbilical arteries were performed before, during, immediately after, and 10, 15, 20, and 30 minutes after the electroconvulsive therapy. Only minor changes were demonstrated in the systolic-

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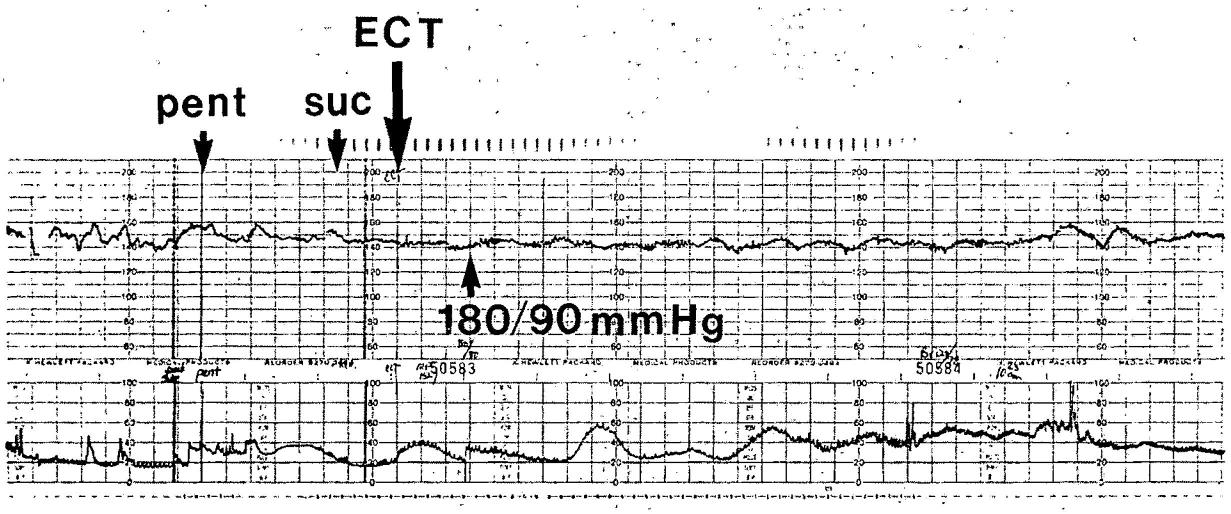

Fig. 1. Continuous cardiotocography during electroconvulsive therapy *(ECT)* at 34 weeks. Note regular uterine contractions and subsequent hypertonic-tetanic contractions. Medications before electroconvulsive therapy *(ECT)* and blood pressure after therapy are noted *(pent,* thiopental sodium; *suc,* succinylcholine) (paper speed, 3 cm/min).

to-diastolic ratio, the pulsability index, and the resistance index of these vessels with higher values immediately after the electroconvulsive therapy. The electroconvulsive therapy at 34 weeks was followed by uterine bleeding to the extent that the patient required observation in the labor and delivery suite for 48 hours. Laboratory test results were normal, and a Kleihauer-Betke test gave negative findings. The bleeding subsided, and she was returned to the psychiatric hospital. A total of seven antepartum electroconvulsive treatments were performed. At 37 weeks the patient presented in spontaneous labor. At admission the cervix was dilated to 1 cm, with the fetal vertex presenting at the spine − 1 station. During labor, because of active uterine bleeding of approximately 800 ml, a transverse, low-segment cesarean section was performed with the patient under spinal anesthesia. A male infant weighing 2704 gm with Apgar scores of 3 and 9 at 1 and 5 minutes, respectively, was delivered. A large retroplacental clot was noted, confirming the diagnosis of abruptio placentae. The patient's postoperative course was unremarkable.

Comment

Previous reports have addressed the apparent safety and efficacy of electroconvulsive therapy for the treatment of depression in pregnancy. These reports failed to demonstrate any complications of labor and delivery or subsequent childhood growth and development.[1, 2] Eclamptic seizures are associated with major maternal and fetal morbidity and mortality. Although a tonic-clonic, grand mal–like seizure is caused by electroconvulsive therapy, it differs from eclampsia in that it is controlled: It is of shorter duration (seconds vs. minutes or longer), the patient is intubated and thus well oxygenated, and the fetus is continuously monitored. A transient increase in blood pressure is known to occur after electroconvulsive therapy. A previous report noting transient maternal hypotension after electroconvulsive therapy in pregnancy was thought to have been due to decreased intravascular volume.[1]

The association of abruptio placentae and hypertensive crises in pregnancy is well documented. We believe the uterine bleeding and uterine hyperstimulation, both manifestations of abruptio placentae, could possibly have been caused by the observed transient marked hypertension. These pathophysiologic events would be similar to those of abruptio placentae noted with acute hypertensive crises and cocaine abuse. Doppler velocimetric studies of the uterine and umbilical arteries might point to a slightly increased impedance to flow in the placental vasculature during electroconvulsive therapy. However, these changes cannot be attributed directly to electroconvulsive therapy itself and may well have been caused by the uterine contractions associated with the electroconvulsive therapy. In any event, although fetal distress did not occur, abruptio placentae in association with transient hypertension noted with electroconvulsive therapy emphasizes the importance of continuous fetal monitoring during this procedure.

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Funic reduction for the management of umbilical cord prolapse

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The current management of umbilical cord prolapse centers on attempts to alleviate the pressure of the presenting part on the cord while preparation for cesarean section is being made. A 10-year experience in which there were vigorous attempts to accomplish safe vaginal delivery after the diagnosis of umbilical cord prolapse is presented. Eight cases of umbilical cord prolapse occurred, a frequency of 1 in 277 deliveries (0.37%), all of which had a normal immediate neonatal outcome. Vaginal delivery was accomplished in seven patients (87.5%); diagnosis was made in two of them when delivery was imminent. Five patients were more remote from delivery and had successful funic reduction (manual replacement of the prolapsed cord). Funic reduction is proposed as a potentially beneficial initial step in the management of umbilical cord prolapse. (Am J Obstet Gynecol 1991;165:654-7.)

Key words: Umbilical cord prolapse, funic reduction, operative obstetrics

The management of umbilical cord prolapse currently centers on attempts to alleviate the pressure of the presenting part on the cord while the patient is being prepared for emergency cesarean section.[1, 2] All but forgotten has been the management of this potential catastrophe by aggressive attempts to achieve vaginal delivery, which was the management plan recommended in the era before attitudes about cesarean section became more liberal. Funic reduction, or manual replacement of the prolapsed umbilical cord, was once considered an appropriate initial step in the management of umbilical cord prolapse.[3, 4] This procedure was sometimes successful, but often delivery after this procedure resulted in an asphyxiated or stillborn neonate.[5] Because of the inability to predict outcome after funic reduction in the era before fetal monitoring, funic reduction was discarded in favor of emergency cesarean section.

The role of funic reduction in the management of umbilical cord prolapse has not been evaluated since continuous intrapartum fetal monitoring has been available. This article discusses my 10-year personal

Table I. Characteristics and outcomes of pregnancies complicated by umbilical cord prolapse

Patient No.	*Age (yr)*	*Gravidity*	*Parity*	*Abortions*	*Gestational age (wk)*	*Pregnancy complications*	*Type of delivery*	*Cervical dilatation at diagnosis (cm)*	*Fetal station at diagnosis*
1	25	1	0	0	31	Premature rupture of membranes	CS	3	−2
2	28	4	3	0	28	Premature rupture of membranes, premature labor, marginal abruptio placentae	SVD	8	+2
3	17	1	0	0	40	None	LMF	Complete	+2
4	26	2	1	0	38	Second twin	LMF	Complete	−2
5	27	2	1	0	41	None	SVD	6	0
6	22	3	1	1	39	None	SVD	6	−1
7	25	6	3	2	40	Previous cesarean, smoker, meconium	SVD	4	−2
8	30	6	2	3	30	Premature labor, drug abuse, fever, hypofibrinogenemia	SVD	4	−1

CVS, Cesarean section; *SVD,* spontaneous vaginal delivery; *LMF,* low-mid forceps delivery.

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experience with umbilical cord prolapse, in which funic reduction was used as an initial management step.

Material and methods

A review was performed that evaluated all private patients whose infants I delivered from July 1, 1980, until June 30, 1990. A total of 2188 deliveries occurred during the study period.

Patients who had had umbilical cord prolapse were reviewed for maternal age, gravidity, parity, cervical dilatation at the time of diagnosis, presence of ruptured membranes when the diagnosis was made, presence of decelerations of the fetal heart rate on fetal monitor before and after the diagnosis, type of delivery, type of anesthesia, gestational age, birth weight, Apgar score, umbilical arterial blood gas values, condition associated with umbilical cord prolapse, and whether funic reduction was attempted.

The diagnosis of umbilical cord prolapse was made by palpating the umbilical cord below the presenting part. When the diagnosis was made and delivery was not thought to be imminent, funic reduction was considered. This was performed by gently lifting the fetal head vaginally and digitally elevating the cord above the widest part of the fetal head in an attempt to place it in the nuchal area. Gentle abdominal pressure in the suprapubic area was simultaneously given to assist elevation of the fetal head and to decrease the chance of oblique or transverse lie. Continuous fetal monitoring was used during and after funic reduction. While this procedure was being performed, preparations for emergency cesarean section were being made.

Results

Eight cases of umbilical cord prolapse occurred, which was an incidence of 1 in 277 (0.37%). Table I describes the patients' characteristics and outcomes. In all cases the neonates were born alive and had normal 5-minute Apgar scores.

The single patient who required cesarean section was not considered to be a candidate for funic reduction, because the cervical dilatation was not felt to be adequate for that procedure. However, because there was no evidence of fetal distress on the fetal monitor, the patient was given an epidural anesthetic for the cesarean section. During the 52 minutes from diagnosis to delivery of the fetus, no abnormalities of the fetal heart rate tracing were noted.

The infants of patients 2 and 3 were delivered vaginally shortly after the diagnosis of umbilical cord prolapse was noted. Funic reduction was not attempted for either of these because, although neither fetus was crowning at the time of diagnosis, vaginal delivery was believed to be near. Patient 2 had severe variable decelerations of the fetal heart rate before the diagnosis of umbilical cord prolapse was made, but patient 3 did not have fetal heart rate decelerations until immediately before delivery.

Funic reduction was successfully accomplished immediately on diagnosis and without an anesthetic in each of the five cases in which it was attempted. None of the patients had ruptured membranes before admission to the hospital. All women in the funic reduction group had previously had one or more vaginal deliveries. The time period between funic reduction and delivery ranged from 14 to 512 minutes. Case 8 was actually a case of funic presentation but function-

			Apgar score		*Umbilical artery blood gas values*		
Funic reduction	*Time from diagnosis to delivery (min)*	*Birth weight (gm)*	*1 min*	*5 min*	*pH*	*P_{CO_2} (mm Hg)*	*Base excess (mEq/L)*
No	52	1389	3	7	7.32	49.2	−1.0
No	8	1049	4	7	7.27	40	−6.5
No	13	2948	8	9	7.27	65	1.5
Yes	14	2070	7	8	7.13	70	−7.7
Yes	26	3544	8	9	7.24	55	−5.0
Yes	80	3289	8	9	7.36	40	−2.0
Yes	322	3884	5	7	7.40	38	−0.7
Yes	512	1219	6	7	7.37	46	1.2

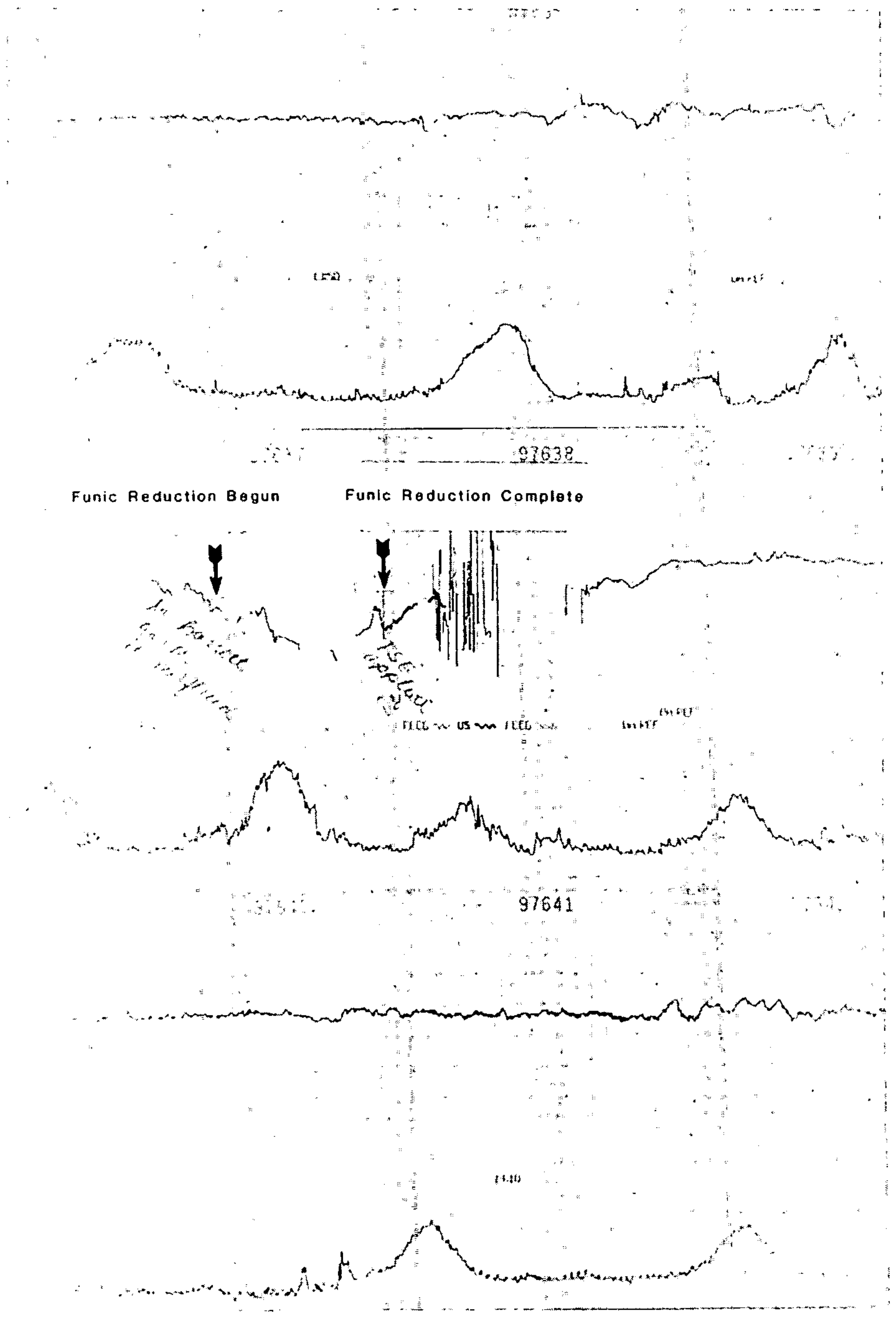

Fig. 1. Fetal monitor tracing before, during, and after funic reduction in case 6.

ally represented the same process as did the other cases.

Typically, a transient deceleration of the fetal heart rate that lasted from 3 to 5 minutes was associated with funic reduction (Fig. 1). In case 4 a second twin had repetitive severe variable decelerations during the 14 minutes between funic reduction and delivery. In case 5 mild variable decelerations occurred later in labor. The other three patients with funic reduction did not have subsequent variable decelerations. In cases 6 and 7 nuchal cords were noted at delivery.

Umbilical cord prolapse was thought to have resulted from iatrogenic causes in cases 6 and 7. In case 6 an amniotomy was performed when the vertex was at the − 1 station and the cervix was 6 cm dilated. In this case the stability of the fetal vertex at the − 1 station was misjudged, and with the leakage of fluid the vertex ascended slightly, allowing cord prolapse to occur.

Funic reduction was promptly performed. In case 7 unplanned rupture of membranes occurred during vaginal examination when the fetal vertex was at the −2 station, allowing cord prolapse.

Comment

In this report the frequency of umbilical cord prolapse was similar to that of other recent reports; however, the 12.5% cesarean section rate associated with umbilical cord prolapse was lower than in other reports, in which the cesarean section rate ranged from 70% to 96%.[2,6,7] In spite of this lower cesarean section rate, no neonatal deaths, no low 5-minute Apgar scores, and no delivery-related morbidity or mortality occurred.

The risk factors noted in this report are similar to those noted in other reports; they include prematurity, twin gestation, and artificial rupture of the membranes when the fetal presenting part was unstable. Breech presentation of the fetus, which is a risk factor for umbilical cord prolapse, was not noted. The absence of breech presentation as a risk factor for umbilical cord prolapse in this report is probably a result of allowing only women whose fetuses present as frank or complete breech to undergo a trial of labor.

Funic reduction was successful in each of the five cases in which it was attempted. In these cases only a relatively short segment of cord had prolapsed, and it is my opinion that funic reduction is much more difficult and perhaps inadvisable if a long (>25 cm) segment of cord is prolapsed. Funic reduction is also contraindicated if there is evidence of fetal distress on the fetal monitor. This procedure requires cervical dilatation that is adequate to place two fingers in the cervix (preferably dilatation ≥4 cm), the ability to elevate the fetal vertex above 0 station, patient cooperation, and gentleness on the part of the physician. It can be performed when membranes are intact. It is accomplished within 2 minutes, and any associated fetal heart rate deceleration is expected to resolve shortly after the procedure is accomplished. When funic reduction is successful, it is important to continuously monitor the fetus for evidence of cord compromise or other problems. While the procedure is being performed, simultaneous preparation for emergency cesarean section must be made. If it is not successful, other steps, such as elevating the fetal head with either manual pressure or bladder filling, as well as the use of tocolytic agents, should be undertaken until cesarean section is performed. If the umbilical cord reduction is successful, then the crisis can be resolved by the time the operating room is made ready for cesarean section, and the maternal and fetal risks of "crash" cesarean section (emergency cesarean section with a general anesthetic) can be avoided.

In this report the experience with funic reduction is clearly limited as a result of the small number of patients involved and the lack of a control group. These limitations are typical of an unplanned event that has a low frequency of occurrence and that is difficult to predict. Nonetheless, the uniformly good outcome in the five cases of umbilical cord prolapse that were managed with funic reduction suggests that, in carefully selected cases of umbilical cord prolapse and with the use of continuous fetal monitoring, this procedure can benefit the mother without undue risk of damage to the fetus. A larger-scale, controlled, modern reevaluation of this procedure to further assess the safety of funic reduction in the management of umbilical cord prolapse is recommended.

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Adverse psychologic consequences of positive cytologic cervical screening

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Cervical cancer is still widely prevalent in the female population. This study explores the relationship of cervical cancer screening, positive versus negative Papanicolaou's test results, and psychologic status among lower-income minority women. All patients were interviewed 3 months after they had received initial test results. One hundred six women with normal Papanicolaou's test results were compared with 118 women who were referred for colposcopic examination for follow-up of positive test results. Women with positive Papanicolaou's-test results showed statistically significant elevations in worries about cancer and impairments in mood, daily activities, sexual interest, and sleep patterns. More fine-grained analyses revealed that the effects of positive results were most pronounced among those women who did not comply with colposcopy (n = 53). These findings suggest that lack of compliance with follow-up may maintain high levels of uncertainty about disease and may interfere with successful psychologic adaptation. Health education targeted to psychologically vulnerable individuals may reduce psychologic distress and enhance compliance. (AM J OBSTET GYNECOL 1991;165:658-62.)

Key words: Cervical screening, psychologic distress, compliance

In 1991 cervical cancer will develop in approximately 13,000 women; 4500 women are expected to die of this disease.[1] Morbidity and mortality rates remain highest among lower-income minority women, who tend to be underrepresented in screening programs for early detection of cervical abnormalities.[2] With improvements in patient and provider education, however, rates of cervical screening are increasing.[3] As a consequence, the detection of cervical intraepithelial neoplasia has risen markedly, particularly among younger women[4] and lower-income minority women.[5] This detection provides an opportunity for the prompt diagnositc follow-up and treatment of precursor lesions, which is essential to prevent invasive cervical cancer.[6]

On the other hand, analyses of the cost-effectiveness of cervical cancer screening programs have underscored the possible adverse psychologic impact on women who receive positive results.[7] It has been argued that the potential psychologic costs of participation might outweigh the medical benefits.[8] There have been anecdotal reports of significant psychosocial consequences of screening[9]; however, only one controlled study has been conducted.[10] In this follow-up of a mass screening for cervical cancer, women with positive results were found to exhibit moderate psychologic distress, but those effects were not long-lasting.[10] The potential of cervical screening to produce psychologic sequelae cannot be ignored because of the possible impact of such sequelae on patient compliance with subsequent screening and diagnostic follow-up.[9, 11] Patient compliance has been cited as a key determinant of the success or failure of cervical cancer detection programs.[12]

This study was designed to evaluate the psychologic effects associated with receiving positive Papanicolaou test results. Cervical cancer worries, mood, tension, and impairment in daily activities, sexual interest, and sleep were examined 3 months after the patients had received Papanicolaou test results. The relationship between adherence (i.e., whether follow-up colposcopic examination was completed) and these psychologic effects also was examined to determine whether such effects persist after cervical cancer has been ruled out. Information regarding the exact nature of psychologic sequelae, as well as the conditions that produce them, is critical if physicians are to manage adverse reactions in their high-risk patients.

Material and methods

Subjects. The subjects were 224 patients seen in the Family Planning Clinic and associated Colposcopy Clinic of Temple University Hospital. These clinics

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Table I. Sociodemographic characteristics of respondents ($N = 224$)

	Screening result					
	All (N = 224)		*Negative (n = 106)*		*Positive (n = 118)*	
Demographic variable	*No.*	*%*	*No.*	*%*	*No.*	*%*
Age group (yr)						
15-24	139	62.7	74	69.8	66	56.4
25-34	67	30.2	28	26.4	39	33.3
35-44	15	6.7	4	3.8	11	9.4
≥45	3	1.4	0	0	1	0.9
Race*						
Black	187	84.1	96	90.6	90	76.3
White or Hispanic	37	15.9	10	9.4	28	23.7
Marital status						
Married	25	11.2	94	88.7	107	90.7
Unmarried	199	88.8	12	11.3	11	9.3
Education†						
Less than high school	64	27.8	15	14.2	48	40.7
High school graduate	103	46.2	52	49.1	45	38.1
More than high school	57	26.0	39	36.8	25	39.1
Employment status*						
Employed	138	61.6	52	49.1	34	28.8
Unemployed	86	38.4	54	50.9	84	71.2

*χ^2: $p < 0.005$.
†χ^2: $p < 0.001$.

serve the predominantly lower-income community of North Philadelphia. One hundred six women had normal Papanicolaou test results that did not require diagnostic follow-up. These women were compared with 118 women who were referred for colposcopic examination for follow-up of positive Papanicolaou test results. Among those with positive results, 65% complied with the scheduled colposcopy appointment. None of these women was found to have invasive cervical cancer. Subjects ranged in age from 15 to 58 years; the average age of the sample patients was 26 years. Demographic characteristics of respondents are shown in Table I. In accordance with the guidelines for human subjects experimentation, all respondents had provided oral consent to participate in this study.

Procedures. About 2 weeks after cervical screening, women received letters informing them about the results of their Papanicolaou tests. Women with positive results were informed that further testing was necessary. They also were notified that an appointment for a colposcopy examination had been scheduled. The date and time of the examination were stated with the clinic telephone number for rescheduling of the appointment, if necessary. Women who did not comply with the colposcopy appointment received a second letter emphasizing the necessity of follow-up and advising them to call the clinic to reschedule.

A brief (10-minute) structured interview was administered by trained telephone interviewers approximately 3 months after the patients had received Papanicolaou test results. This time period was selected to allow sufficient time for the scheduled diagnostic follow-up to rule out cervical cancer. All survey questions were forced-choice with Likert scale responses. Interviewers were blind to subjects' Papanicolaou test results. The response rate for these interviews was 70%; 26% of women could not be reached after four attempts, 2% of women were ineligible as a result of a language barrier, and 2% refused to be interviewed. The resulting sample of respondents included 224 women.

Measurements. Questions assessed respondents' sociodemographic characteristics (age, race, marital status, education, and employment status) and screening-sexual history (number of previous Papanicolaou tests, number of different sexual partners in the previous 3 months, and history of sexually transmitted disease). The following psychologic variables also were assessed: (1) current frequency of worry about cervical cancer (1 was never and 4 was often), (2) current impairment in daily activities as a result of cervical cancer worries (1 was never and 4 was often), (3) tension in the past month (1 was never and 4 was often), (4) mood in the past month (1 was very good and 5 was very bad), (5) sexual interest (1 was not impaired and 4 was impaired), and (6) sleep patterns (1 was not impaired and 4 was impaired). Mood and tension items were adapted from the Mental Health Inventory.[13] Other items were adapted from previous work on the psychologic impact of abnormal findings of mammography.[14]

Results

Demographic characteristics. As shown in Table I approximately 63% of respondents were aged 15 to 24

Table II. Psychologic responses by screening result

Psychologic response	*Screening result*			
	Negative (n = 106)		*Positive (n = 118)*	
	No.	*%*	*No.*	*%*
Cervical cancer worries				
Often	15	14.1	35	29.8
Sometimes	31	29.5	41	34.5
Never or rarely	60	56.4	42	35.7
Impairment in activities				
Often	3	2.8	10	8.3
Sometimes	12	11.4	25	21.2
Never or rarely	91	85.8	83	70.5
Tension				
Often	25	23.5	35	29.8
Sometimes	55	51.9	45	38.2
Never or rarely	26	24.6	38	32.0
Mood				
Very bad or mostly bad	6	5.7	28	23.5
Some good or some bad	67	63.0	58	49.3
Very good or mostly good	33	31.3	32	27.2
Sexual interest				
Impaired	33	31.4	59	50.0
Not impaired	73	68.6	59	50.0
Sleeping patterns				
Impaired	27	25.7	48	40.5
Not impaired	79	74.3	70	59.5

and 30% were aged 25 to 34. Eighty-four percent of respondents were black and only 11% were married. Most respondents (72%) had had at least a high school education, and 61% of respondents were currently employed. However, the lack of completed high school education and employment may, in part, reflect the young age of some of the respondents. According to χ^2 tests women who had positive test results were significantly more likely to be unemployed ($\chi^2 = 8.8$, $p < 0.005$) and to have had less formal education ($\chi^2 = 20.2$, $p < 0.001$). Women with negative results were significantly more likely to be black ($\chi^2 = 7.1$, $p < 0.005$).

Screening and sexual history. The majority of women reported that they had had previous Papanicolaou tests: 41% had had >10, 35% had had 5 to 10, and 34% had had <5. Ninety-two percent of women reported that they had had either no sexual partner or one sexual partner in the previous 3 months. Women in the different result groups (negative or positive result) did not differ significantly on these two characteristics. These groups did differ, however, with respect to their history of sexually transmitted diseases; 70% of women with positive results and 48% of women with negative results reported a history of at least one sexually transmitted disease ($\chi 2 = 11.0$, $p < 0.001$).

Factors associated with test result. Proportions of women with negative and positive test results who reported psychologic distress are shown in Table II. Thirty percent of the women with positive results reported that they often worried about cervical cancer, as compared with only 14% of women with negative results. In addition, 25% of women with positive results reported some impairment in their daily activities as a result of these worries, as compared with 11% of women with negative results. Fifty percent of women with positive results reported impairment in sexual interest and 40% had sleep disturbance, as compared with 31% and 26% respectively, among control patients. Student t tests were conducted to examine whether average ratings of psychologic responses differed significantly for women in the different test result groups. Women with positive results reported significantly more frequent worries about cervical cancer ($t = -3.01$, $p = 0.002$), more impairment in daily activities as a result of these worries ($t = -2.09$, $p = 0.02$), worse mood ($t = -2.20$, $p = 0.01$), more impairment in sexual interest ($t = -2.31$, $p = 0.01$), and more impairment in sleep ($t = -1.81$, $p = 0.04$). Between-group differences in tension were not statistically significant.

Multivariate analyses. Multivariate regression analyses were conducted for each of the five psychologic outcomes that exhibited significant bivariate associations with the test result. Demographic and sexual-screening history variables were included with the test result in the regression analyses to control for potential confounding influences on psychologic outcomes. To examine whether the effects of test results on psychologic outcomes were independent of compliance with follow-up, two-level index variables were created: (1) negative result versus positive result with subsequent colposcopy; (2) negative result versus positive result without subsequent colposcopy.

Women who had positive results but had not complied with colposcopy had significantly more frequent worries ($r = -0.21$, $p = 0.002$), more impairment in daily activities ($r = -0.20$, $p = 0.003$), worse moods ($r = -0.17$, $p = 0.01$), and more impairment in sleep ($r = -0.15$, $p = 0.03$) and in sexual interest ($r = -0.13$, $p = 0.01$) when compared with women who had negative results. By contrast, women who had positive test results but had undergone colposcopy differed from women with negative results in only two of these characteristics: impairment in daily activities ($r = -0.10$, $p = 0.004$) and in sleep ($r = -0.16$, $p = 0.03$). The effects of positive test results on psychologic responses were independent of all demographic and sexual-screening history confounder variables examined.

Comment

This study examined the psychologic impact of receiving positive results after cervical cancer screening in a population of minority women. Substantial and generalized negative psychologic consequences of a positive Papanicolaou test were observed 3 months after the patients had received test results. Not surprisingly, women with positive results exhibited heightened worries about cervical cancer and worsened moods when compared with women who had negative results. In fact, more than one third of women with positive results reported that they often worried about cervical cancer. In addition, these women reported greater impairment in daily activities and in sexual interest and sleep patterns when compared with women who had negative results.

More in-depth analyses revealed that these effects depended on the follow-up status of women who had positive test results. Women who had undergone colposcopy (65% of the sample) did not exhibit heightened worry or disturbance in mood or sexual interest when compared with women who had negative results. Completion of diagnostic follow-up may have reduced the uncertainty and uncontrollability surrounding a positive diagnosis, thereby ameliorating the negative psychologic sequelae of screening. In contrast, women who did not comply with colposcopy exhibited persistent distress and impairment. By not adhering to the recommended regimen, these women did not avail themselves of the opportunity to reduce distress by learning about the efficacy of early detection and treatment. This decision may create a vicious cycle in which failure to comply sustains uncertainty about disease and accompanying distress[15] that in turn contributes to delay in follow-up.

These results have implications for how health care professionals manage individuals in the course of cervical screening. Previous research suggests that proper education and counseling of women with positive Papanicolaou tests can improve psychologic status and can enhance compliance with follow-up. In a recent study, for example, women who received an informational leaflet along with postal notification of positive Papanicolaou test results were less anxious than were women who did not receive this additional information.[16] Such written communications about positive test results should highlight specific areas of patient concerns that may function as barriers to compliance, including cancer fears, loss of sexual-reproductive function, and aversion to medical procedures.[9] Indeed, mailings of written materials that bolster positive attitudes and address such barriers to follow-up have been shown to be a cost-effective mechanism for improving compliance.[17] This strategy may be supplemented profitably by more intensive efforts (e.g., structured telephone calls) for women who remain recalcitrant to follow-up. These educational messages should be targeted to women who are most likely to be lost to follow-up (those who are younger, unmarried, or of lower socioeconomic status.[18]

This study suffers from two main limitations. First, psychologic parameters were measured at a single time point 3 months after the patients were notified of these results. Therefore we cannot determine whether psychologic distress among noncompliers resulted from the uncertainty of their diagnoses or whether excessive psychologic distress predated and contributed to noncompliance. Prospective studies should evaluate, in a more fine-grained manner, patterns of patients' psychologic response and behavior during several key phases of the screening process. These include measures obtained before screening, while anticipating results, after notification, and during and after diagnostic follow-up and treatment. Second, the study population was predominantly younger, black, and less well educated. These findings therefore may not apply to the larger cervical screening population. On the other hand, this subpopulation constitutes the greatest risk group for invasive disease.[3] Ongoing research will help to pinpoint the role of psychosocial variables in contributing to avoidable cervical cancer morbidity and mortality in these high-risk populations.

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Prospective evaluation of maternal serum human chorionic gonadotropin levels in 3428 pregnancies

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As part of a multicenter prospective study, second-trimester human chorionic gonadotropin and α-fetoprotein concentrations were evaluated. Data included maternal age, human chorionic gonadotropin level, α-fetoprotein level, weight, race, and pregnancy outcome of 3428 pregnancies at between 15 and 20 weeks' gestation. The results of the study indicate that human chorionic gonadotropin levels decrease as maternal weight increases, that weight-adjusted human chorionic gonadotropin levels for Oriental and black women are higher than for white or Hispanic women, and that twin pregnancies have higher human chorionic gonadotropin levels than singleton pregnancies. Of 255 pregnancies that did not have normal outcomes, 54 (21.2%) had human chorionic gonadotropin levels >2.0 multiples of the median and 26 (10.2%) had α-fetoprotein levels >2.5 multiples of the median. Of 11 pregnancies with fetal aneuploidy, 6 (54.5%) had human chorionic gonadotropin levels >2.0 multiples of the median. It is concluded that in human chorionic gonadotropin screening programs for fetal Down syndrome, weight and race adjustments are necessary for accurate risk assessment. (AM J OBSTET GYNECOL 1991;165:663-7.)

Key words: Human chorionic gonadotropin, α-fetoprotein, Down syndrome, aneuploidy

The decision to prenatally screen pregnant women for fetal Down syndrome and other forms of aneuploidy has traditionally been made on the basis of maternal age. In recent years the use of combined maternal age and maternal serum α-fetoprotein concentrations (AFP) has become routine; it has recently been estimated that approximately 1 million pregnancies are screened according to these parameters.[1] There is widespread use of maternal serum AFP in spite of a combined maternal serum AFP and age detection rate of <40%.[2] In 1987 Bogart et al.[3] reported an association between abnormal human chorionic gonadotropin (hCG) levels and pregnancies with fetal aneuploidy. In that and subsequent studies it has been shown that elevated levels of hCG are associated with Down syndrome and other aneuploidies and that very low levels of hCG are associated with trisomy 18 syndrome pregnancies.[3-10]

All studies published so far are retrospective and include limited numbers of normal control pregnancies for comparison. Given the limits of the retrospective studies, the most effective proposed screening procedure is that described by Petrocik et al.[6] in which multiple regression probit analysis of hCG, maternal age, and maternal serum AFP values yielded a detection rate of 68.3% for 38 Down syndrome pregnancies, with a false-positive rate of 5%. Wald et al.[5] have published the largest data base, which includes 77 Down syndrome pregnancies with 60% detection at a false-positive rate of 6.7%. The false-positive rate was reduced to 4.7% when unconjugated estriol evaluation was included.

Our multicenter collaborative study was designed as a nonintervening prospective study to collect data on maternal serum hCG levels in a population that was already undergoing maternal serum AFP screening for neural tube defects. We collected data on hCG levels, maternal serum AFP levels, race, weight, age, pregnancy outcome, and karyotype results when amniocentesis was performed for other clinical indications.

Material and methods

Maternal serum samples were collected between 15 and 20 weeks' gestation as part of routine maternal serum AFP screening programs in seven geographically diverse areas: Arizona, California, Georgia, South Carolina, Texas, Virginia, and Washington. The number of patients enrolled in the study for each geographic region is presented in Table I. Patients were inter-

From the University of California, San Diego,[a] University of Virginia,[b] University of South Carolina,[c] Arizona Institute for Genetics, Chandler,[d] Group Health Cooperative, Renton, Washington,[e] University of California, Los Angeles,[f] Emory University,[g] Lackland Air Force Base, Texas,[h] and Hybritech Inc., La Jolla.[i]

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Table I. Numbers of patients from various geographic regions

Location	*No. of patients*
Arizona	970
California	117
Georgia	626
South Carolina	478
Texas	588
Virginia	248
Washington	401
TOTAL	3428

viewed at the time of serum collection for information on maternal age, weight, race, family history of neural tube defects, and diabetes. Pregnancy outcome data were collected from patient records. These records include both reporting of outcome by patients and physician notes of pregnancy complications and abnormalities. Because there was no postdelivery follow-up by physicians, the possibility of underreporting minor abnormalities and abnormalities that are not apparent at birth cannot be ruled out. If amniocentesis was performed, karyotype results were also recorded. Gestational ages were determined by ultrasonographic evaluation when available or from the date of the mother's last menstrual period. Data were collected from 3428 pregnancies.

The β-hCG and AFP levels were assayed with commercially available enzyme immunoassays (Tandem-E hCG and Tandem-E AFP kits, Hybritech, San Diego). AFP assays were performed according to kit instructions. Kit standards for hCG are calibrated against the World Health Organization second international standard. Because of the high concentration of maternal serum hCG in the second trimester, serum samples were diluted in the diluent provided with the kits. Samples were diluted by hand or with a semiautomated diluter at 1:200 or 1:231. If an initial value was >375 mIU/ml or <10 mIU/ml, the sample was assayed again at appropriate dilutions. Samples were assayed twice, and any sample with a coefficient of variation >10% was assayed again. Interassay variation among all centers was 10.2%. Assay results from individual centers were further analyzed with Levene's test for equality of variance, Welch's test for equality of means, Bonferroni's test of significance between pairs of means, and the Student—Newman-Keuls multiple mean comparison test to confirm that the assay results could be pooled. These tests confirmed that the samples collected and assayed at the various locations could be pooled and analyzed as a group.

Results

Of the 3173 pregnancies that resulted in a normal outcome (defined as no birth defects or complications of pregnancy), 2505 mothers were white, 310 were black, 185 were Hispanic, 93 were Oriental, and 80 were of unidentified or other racial or ethnic groups. Table II describes the major categories of pregnancies that were not classified as normal. Of the 255 pregnancies that did not have normal outcomes, 112 involved abnormalities that were individually rarely observed or had not involved abnormal hCG or AFP levels. Percentages with elevated hCG levels (>2.0 multiples of the median value from normal pregnancies) or elevated maternal serum AFP levels (>2.5 multiples of the median) are also listed.

Median hCG levels for weeks 15 to 20 were calculated for the normal-outcome pregnancies of 2505 white women and are presented in Table III. Table III also presents the median hCG levels for normal-outcome pregnancies for black, Hispanic, and Oriental women.

Multiples of the median values for normal pregnancies in white women decreased as maternal weight increased. The following equation describes multiples of the median (MoM) as a function of maternal weight: MoM (corrected) = MoM/F, where $F = e^{[0.5145 - (0.00372 \times \text{weight in pounds})]}$.

The median weight of white women from Virginia (144 pounds) was elevated ($p = 0.064$) and the median weight of white women from Washington (135 pounds) was significantly less ($p = 0.006$) than the overall median (139 pounds).

In this study the median weights of white, Hispanic, Oriental, and black women were significantly different (Table IV). Statistical analysis indicated that there was no significant difference between the hCG levels of whites and Hispanics ($p = 0.56$) or whites and blacks ($p = 0.77$); however, the weight-adjusted multiples of the median of blacks were 9.8% higher than those of whites ($p = 0.008$) (Fig. 1). There was a significant difference between the hCG levels of whites and Orientals ($p = 0.0001$), and the weight-adjusted multiples of the median for Orientals were 16% higher ($p = 0.013$) (Fig. 1).

There were 33 twin pregnancies with normal outcomes and three twin pregnancies with abnormal outcomes (1 hydrops, 1 omphalocele, and 1 fetal death). The weight-adjusted median hCG level for the normal-outcome twins was 1.48 multiples of the normal singleton white weight-adjusted median hCG level, which is significantly elevated ($p = 0.002$).

The 11 pregnancies found to have chromosome abnormalities are listed in Table V. Six of the 11 had weight-adjusted hCG levels >2.0 multiples of the median (54.5%), including four of six pregnancies with Down syndrome (66.7%). The case of trisomy 20 mosaicism resulted in a normal female infant. Blood chromosome analysis did not reveal any trisomy 20 cells. This case most likely reflects confined mosaicism. Of

Table II. Weight-adjusted hCG from abnormal pregnancy outcomes

Outcome	No.	hCG > 2.0 MoM		AFP > 2.5 MoM	
		No.	%	No.	%
Fetal death	43	5	11.6	9	20.9
CPD–fetal distress	8	5	62.5	0	0
Prematurity–low birth weight	45	13	28.8	4	6.2
Aneuploidy	11	6	54.5	1	9.1
Twins (normal)	33	12	36.4	9	27.3
Twins (abnormal)	3	0	0	1	33.3
Others*	112	17	15.2	2	1.8
TOTAL	255	58	22.7	26	10.2

MoM, Multiples of the median; *CPD,* cephalopelvic disproportion.

*All other pregnancies reported to be abnormal, including pregnancies involving omphalocele, dysplastic kidneys, gestational diabetes, hydrops, oligohydramnios, polyhydramnios, Potter's syndrome, cleft lip, and clubfoot.

Table III. Median hCG values and number of pregnancies

Weeks' gestation	White		Black		Hispanic		Oriental	
	hCG value	No. of pregnancies	hCG value	No. of pregnancies	hCG value	No. of pregnancies	hCG value	No. of pregnancies
15	32.80	237	34.77	37	41.63	10	41.60	7
16	27.49	1222	26.65	112	26.20	94	38.12	45
17	22.41	633	24.63	58	24.30	42	28.00	19
18	20.21	261	22.51	61	19.82	16	29.10	13
19	20.21	91	18.70	28	18.31	12	21.04	9
20	18.13	61	15.12	14	27.80	11	—	

hCG values are before weight adjustment.

the white women with pregnancies having normal outcomes, 9.82% had weight-adjusted hCG levels >2.0 multiples of the median.

The median age of patients in this study was 28 years. There was no correlation between hCG values and maternal age (correlation coefficient = 0.028). There were insufficient data to determine any correlation between maternal diabetes and hCG levels.

Table IV. Median weights

Group	Median weight (lb)	No.	p*
White	139	2505	—
Hispanic	133	180	0.008
Oriental	115	90	0.0001
Black	147	310	0.0001

*Significance between median weights of white women and other groups ($p < 0.05$ is considered to be significant).

Comment

The results of this study indicate that maternal weight adjustment is needed in routine hCG screening for fetal aneuploidy. The weight-adjustment formula we developed for hCG values takes the same form previously described for maternal weight adjustment for maternal serum AFP evaluation[11] showing a decrease in median hCG levels with increasing maternal weight.

After weight adjustment, hCG levels from the normal singleton pregnancies of Oriental women were 16% higher than those for white women. However, because we evaluated hCG levels in the pregnancies of only 93 Oriental women with normal outcomes, further studies with larger numbers of Oriental women are needed before an accurate adjustment for Oriental-race factor can be calculated. It is interesting to note that the incidence of trophoblastic disease is greater in Orientals than in whites.[12] Whether the increased level of hCG contributes to or reflects a significant risk factor for trophoblastic disease should be the subject of further study.

Our results confirm the report by Petrocik et al.[6] that there is no statistically significant difference in hCG levels between white and black patients; however, this conflicts with a recently reported study in which significantly different levels were observed between these two racial groups.[13] On the other hand, we did observe a significant difference between the *weight-adjusted* multiples of the median for blacks and whites, with blacks having 9.8% higher weight-adjusted hCG levels. Although our data are taken from only 310 pregnant black women, it appears that the placenta of black

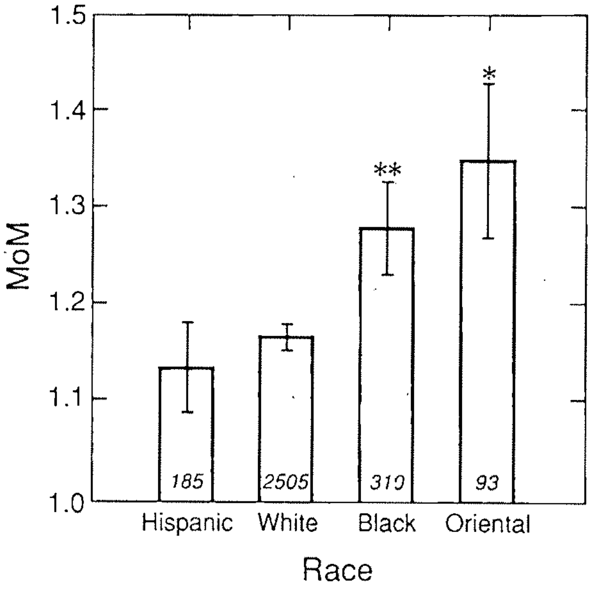

Fig. 1. Weight-adjusted multiples of the median (mean ± SEM) of different racial groups. Numbers within bars equal number of patients. *One asterisk,* Significance between white and Oriental women ($p = 0.01$); *two asterisks,* significance between white and black women ($p = 0.008$).

women produces 9.8% more hCG than the placenta of white women. Whether the increased amount of hCG is due to increased placenta size or increased production cannot be determined from this study. It is worth noting that the hCG levels recently reported for blacks by Simpson et al.,[13] using the same assay system that was used in our study, are nearly the same as we report for whites. It appears that the median hCG levels for whites in the study by Simpson et al., are less than the levels we observed. Such geographic variation in hCG levels in the pregnancies with normal outcomes of white women have previously been reported by Bartels et al.[14] and most likely reflect differences in maternal weight rather than a true difference in hCG production by the placenta. In this regard we observed a significantly lower weight in women enrolled in this study in Washington and a higher weight in women from Virginia. We believe that the use of weight-adjustment formulas is critical to accurate assessment of maternal hCG levels and that the use of such formulas is appropriate both for comparative studies and for clinical evaluation of Down syndrome risk. Additional factors such as smoking may also affect hCG levels[15, 16] and result in differences among subgroups within the population.

Although there is no documented difference in the incidence of fetal aneuploidy between different racial groups, screening programs that do not account for racial differences in weight, hCG production, and perhaps smoking habits may result in different detection and false-positive rates.

Table V. Weight-adjusted hCG values for aneuploid pregnancies

Abnormality	*Gestation (wk)*	*hCG (IU/ml)*	*MoM*
47,+21	16	31.0	1.13
47,+21	16	118.3	4.29
47,+21	18	41.3	2.07
47,+21	19.5	72.6	3.59
47,+21	20.7	39.8	2.20
47,+21	20.5	10.5	0.58
47,+18	16	10.0	0.36
47,+18	20	12.0	0.59
47,+13	16	31.0	1.13
45, X	16	106.8	3.89
46,XX/47,+20*	16	76.4	2.78

MoM, Multiples of the median.
*See article text.

Our results indicate no correlation between maternal age and hCG levels; these results are consistent with previous reports[5, 6] and indicate that age and hCG levels are independent factors useful in screening for fetal aneuploidy.

Because of the increased placenta volume in twin as opposed to singleton pregnancies, the level of maternal serum hCG has been reported to be higher in twin pregnancies.[17] In our group of 33 twin pregnancies with normal outcomes, weight-adjusted hCG levels were significantly elevated ($p = 0.001$), with the median weight-adjusted hCG level approximately 1.5 times the singleton median.

In our study 6 of 11 pregnancies (54.5%) with fetal aneuploidy had abnormal hCG levels, including four of six pregnancies (66.7%) with Down syndrome (Table IV). These results are consistent with previous reports[3-10]; however, because of the limited number of cases of aneuploidy in this study, the statistical validity of the detection percentage is unreliable.

To more accurately evaluate the relationship between hCG, AFP, age, and aneuploidy, we have evaluated 49 Down syndrome pregnancies between 15 and 20 weeks' gestation using a probability risk calculation similar to that of Petrocik et al.[6] We observed a detection rate of 65.3%, with a false-positive rate of 7.85% (data not presented). This is quite similar to the findings of Petrocik et al. of approximately 68% detection at a 5% false-positive rate. Our false-positive rate is somewhat higher but is primarily based on gestational age estimation from the date of the mother's last menstrual period. It is well documented that approximately one third of women with abnormal maternal serum AFP levels have incorrect gestational age estimation when date of last menstrual period is compared with ultrasonographic evaluation.[18] Assuming that there is a similar occurrence in our study of one third of the women with abnormal hCG levels having incorrect gestational age estimates, our false-positive rate would drop to approximately 5.2% after ultrasonographic evaluation. Had this been an intervening study 7.8% of the women would have been referred for ultrasonographic evaluation, but only 5.2% would have had amniocentesis.

Screening programs can be expected to lead to the detection of approximately 65% of fetal aneuploidy, including Down syndrome, within the approximately 5% to 7% of women offered amniocentesis when calculation of combined-risk rates include evaluation of maternal age and hCG level. The findings of Wald et al.[5] and Petrocik et al.[6] indicate that addition of maternal serum AFP evaluation to hCG and age only minimally increases detection of fetal aneuploidy; however, the value of maternal serum AFP in detecting other types of abnormalities is well known. The addition of other biochemical parameters such as the gonadotropin α-subunit,[3, 4] unconjugated estriol,[5, 19] and pregnancy-specific β_1-glycoprotein (SP1)[20] for increasing detection of fetal aneuploidy have been proposed. There are conflicting claims regarding the value of unconjugated estriol[19, 21] and SP1,[14, 20, 22] and the findings of Bogart et al.[3, 4] on the free α-subunit have not been independently verified. Thus the value of these biochemical tests in addition to or as a replacement for maternal serum AFP in age-hCG-AFP risk evaluation for fetal aneuploidy remains to be proved. Decisions regarding the incorporation of one or more of the alternative biochemical tests depend on judgment of cost, accuracy, consistency, available assay systems, and ease of use. At this time there is insufficient evidence to determine which protocol is most efficacious. Regardless of conflicting claims regarding the addition of alternative biochemical tests, all screening protocols primarily depend on hCG evaluation. Thus it is important to establish baseline data on race differences, to establish weight-adjustment formulas, and to begin recognizing other pregnancy complications that are associated with elevated levels of hCG.

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Fetal atrioventricular and outflow tract flow velocity waveforms during normal second half of pregnancy

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Maximum flow velocity waveforms at atrioventricular and outflow tract level were studied longitudinally in 40 fetuses from 18 to 38 weeks' gestation at 3- to 4-week intervals. Flow velocity parameters studied were peak systolic and time-averaged velocity and acceleration time at outflow tract level and time-averaged velocity, peak E-wave and peak A-wave velocities, and E/A ratio at atrioventricular level. An increase of all flow parameters with advancing gestational age was found. Aortic parameters were higher than pulmonary ones. Time-averaged, peak E-wave, and peak A-wave velocities were higher at tricuspid level than at mitral level, whereas E/A ratios were higher at mitral level. Outflow tract parameters showed no correlation with period time. A positive correlation for peak E-wave velocity and E/A ratio, and a negative correlation for time-averaged and peak A-wave velocity was found with period time. These results suggest that the normal second half of pregnancy is characterized by rising ventricular stroke volume and output and reduction in ventricular afterload. The left ventricular afterload seems to be lower than the right ventricular afterload. Atrioventricular flow velocities are heart rate dependent. (AM J OBSTET GYNECOL 1991;165:668-74.)

Key words: Fetal cardiac blood flow, Doppler ultrasonography, fetal heart rate

Transabdominal Doppler techniques can be used to determine flow velocities at the level of the human fetal heart from about 16 weeks' gestation onward.[1, 2] This allows information about fetal cardiac function to be obtained in a noninvasive way. Both cross-sectional and serial measurements of cardiac flow velocities at different gestational ages have been performed by several centers.[1-7] However, to our knowledge a detailed analysis of waveforms collected from all four fetal cardiac valves has never been carried out in a longitudinal setting. Moreover, few data have been published concerning the influence of variables such as fetal heart rate on fetal cardiac flow velocity waveforms.

The objective of the present study was twofold: (1) to establish the distribution of peak systolic velocity, acceleration time, and time-averaged velocity in the fetal cardiac outflow tract (ascending aorta and pulmonary artery) and time-averaged velocity, peak E-wave velocity, peak A-wave velocity, and E/A ratio at atrioventricular level (mitral valve and tricuspid valve) between 18 and 38 weeks' gestation; (2) to determine whether there is fetal heart rate dependency for one or more of the above parameters within the physiologic fetal heart rate range (i.e., 110 to 160 beats/min) and, if so, to establish the relationship between fetal period time (the reciprocal of heart rate) and the parameter concerned.

Patients and methods

A total of 40 women with normal singleton pregnancies consented to participate in the study. The study protocol was approved by the Hospital Ethics Committee. Fetal cardiac Doppler examinations were carried out at 3- to 4-week intervals between 18 and 38 weeks' gestation. The first examination was performed between 18 and 22 weeks' gestation.

Normal pregnancy was defined by a normal fetal biparietal diameter and birth weight between the 5th and 95th percentiles according to Kloosterman's tables, corrected for maternal parity and fetal sex.[8] The pregnancy duration was determined from the last menstrual period and confirmed by ultrasonographic measurements of the biparietal diameter between 14 and 18 weeks' gestation. The median maternal age was 29 years (range, 18 to 41 years), and the median parity was one (range, 0 to 6).

A combined mechanical sector scanner and pulsed Doppler system (CV 400, Diasonics, Milpitas, Calif.) with a carrier frequency of 3.5 and 3.0 MHz was used for blood flow velocity measurements in the ascending aorta, in the pulmonary artery, and at the mitral and tricuspid valves. The sector scanner operates at power outputs <100 mW/cm² spatial peak/temporal average

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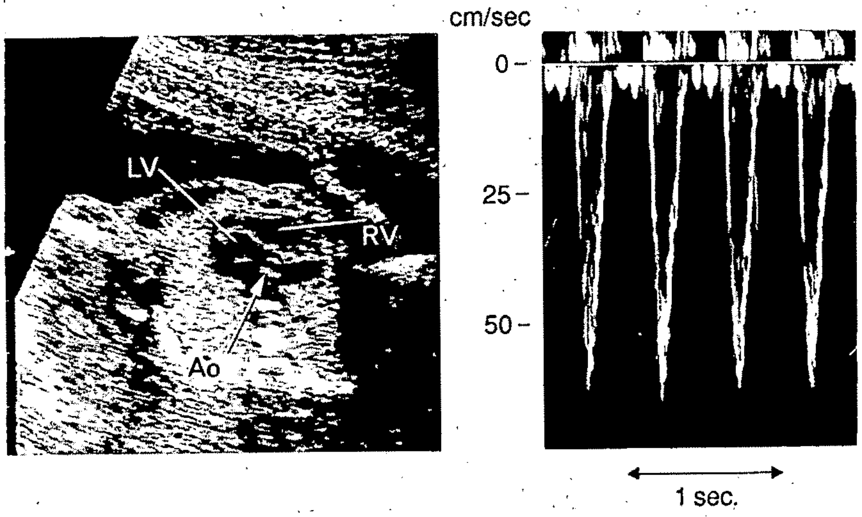

Fig. 1. Two-dimensional five-chamber view of the fetal heart, with Doppler flow velocity waveform tracing from ascending aorta *(AO)* at 21 weeks' gestation. *LV*, Left ventricle; *RV*, right ventricle.

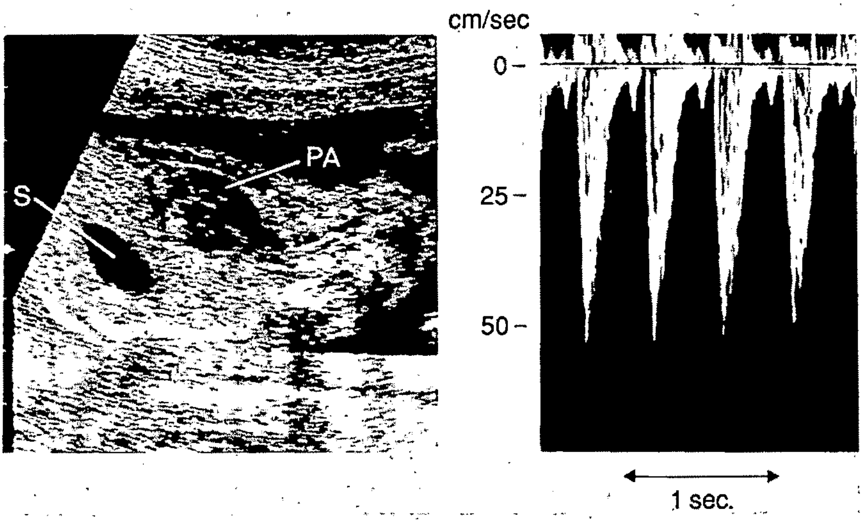

Fig. 2. Two-dimensional short-axis view of fetal heart with Doppler flow velocity waveform tracing from pulmonary artery *(PA)* at 21 weeks' gestation. *S*, Stomach.

in both imaging and Doppler modes by manufacturer's specifications. Two-dimensional imaging was used to ensure the correct position of the pulsed Doppler gate, both before and after each Doppler tracing was obtained. Maximum flow velocity waveforms from the ascending aorta were recorded from the five-chamber view (Fig. 1). Maximum flow velocity waveforms from the pulmonary artery were collected from the conventional short-axis view (Fig. 2). Maximum flow velocity waveforms from the mitral and tricuspid valve were recorded from the four-chamber view (Fig. 3). At all sites the Doppler sample volume was placed immediately distal to the valve leaflets. The Doppler sample volume length ranged between 0.1 and 0.4 cm. Doppler tracings were accepted when the angle between the Doppler cursor and the assumed direction of flow was ≤10 degrees. Doppler studies were performed by one examiner (K.v.d.M.). All blood flow velocity waveforms were obtained during fetal apnea and stored on videotape. From hard copies the analysis of four consecutive waveforms was carried out with a microcomputer (M240, Olivetti, Scaramagno, Italy) linked to a graphics tablet. The following parameters were calculated: (1) in the two outflow-tract vessels—peak systolic velocity (centimeters per second), acceleration time (milliseconds), time-averaged velocity (centimeters per second), the ratios of these parameters at both sites, and the period time (milliseconds); (2) at atrioventricular level—time-averaged velocity (centimeters per second), peak velocities of E wave and A wave (centimeters

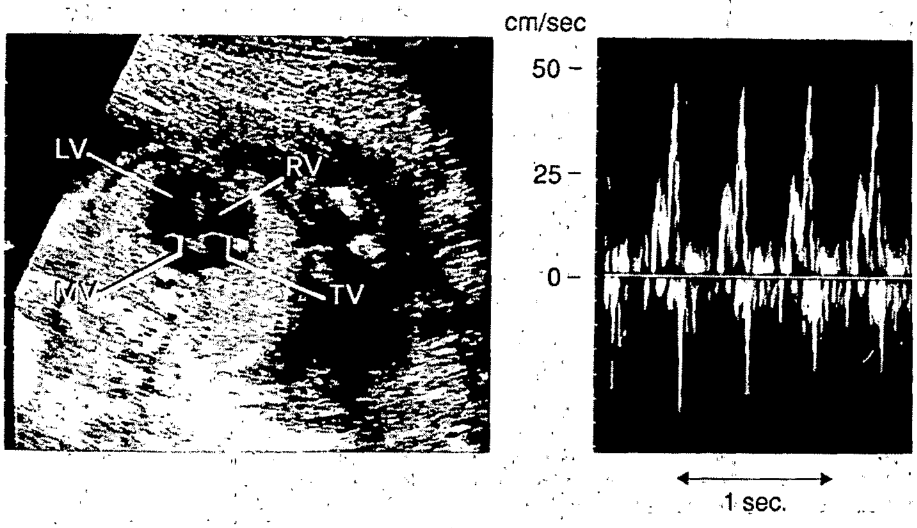

Fig. 3. Two-dimensional four-chamber view of fetal heart with Doppler flow velocity waveform tracing at tricuspid valve level at 21 weeks' gestation. *MV*, Mitral valve.

per second), E/A ratio, period time (milliseconds), and the ratio of tricuspid to mitral time-averaged velocity.

Peak velocities were measured from the 0 line to the highest point of the Doppler velocity peak. Acceleration time was measured as the time between the onset of ejection and the peak of velocity.[9] Time-averaged velocity was calculated as area under the curve divided by period time.

All Doppler studies were performed 2 hours after breakfast or lunch, with the patient in a semirecumbent position. The duration of one investigation never exceeded 30 minutes.

Statistical analysis included assessment of the relationship between all parameters studied and gestational age by repeated measurements analysis of variance, for which the BMDP program 5V (BMDP statistical software manual, volume 11, 1988, University of California Press, Berkeley) was used. The p values were determined by means of the sign test. The relationship between each parameter and fetal heart rate was studied as follows. For every patient the available data were interpolated in a linear fashion to a gestational age of 30 weeks, to rule out the influence of gestational age on the parameters. The reason for selecting 30 weeks was determined by the range of gestational ages (18 to 38 weeks) studied. For each adjusted parameter and period time the correlation coefficient was then calculated, and the corresponding p value was established. Left-to-right differences of the means per fetus were assessed with the paired t test. The lower level of statistical significance was set at 0.05.

Results

The success rate for obtaining good-quality flow velocity waveforms was 99% for the ascending aorta, 97% for the pulmonary artery, 99% for the mitral valve, and 97% for the tricuspid valve. In 4% of the mitral valve measurements and in 18% of the tricuspid valve measurements, it was not possible to separate E and A waves. This was more common with high-normal fetal heart rates. In these cases analysis of the waveform included only time-averaged and peak A-wave velocities. In each patient it was possible to obtain at least four serial recordings at all cardiac levels during the study period. On average, five investigations were performed in each pregnancy. Serial recordings of one particular valve or vessel were never longer than 6 weeks apart. In total, 233 studies were performed at a gestational age of 28.7 ± 5.8 weeks (mean ± SD). The relationship between any of the flow velocity waveform parameters and gestational age was approximately linear for each patient. It also appeared that the regression lines, by which the relation between parameter and gestational age for each of the patients could be described, differed significantly from patient to patient with respect to both the intercept and the slope. Therefore the following statistical model was adopted as the basis for the calculation of approximate 95% prediction bounds for each of the parameters, when the gestational age is known:

$$y = a + b(x - 30) + e$$

where y is the parameter concerned, x is the gestational age (weeks), a is the intercept of the regression line, b is the slope of the regression line, and e is an error term.

Thus for each of the parameters a regression line was constructed with its 2.5% and 97.5% confidence limits. The values for a, b, and e for each flow velocity waveform parameter, as well as the p values of the slopes, are listed in Table I.

Table I. Absolute values and standard errors of parameters determining regression lines for all flow velocity parameters with gestational age according to general formula $y = a + b\ (x - 30) + e$

	a	*b*	*Sa*	*Sb*	*Error (e)*	*p Value*
Pulmonary artery						
Peak systolic velocity (cm/sec)	60.1	1.18	6.9	0.06	10.4	<0.0001
Time-averaged velocity (cm/sec)	21.7	0.53	1.0	0.02	2.4	<0.0001
Acceleration time (msec)	51.7	1.96	25.9	0.25	19.8	<0.0001
Ascending aorta						
Peak systolic velocity (cm/sec)	74.8	1.35	13.9	0.08	21.8	<0.0001
Time-averaged velocity (cm/sec)	24.1	0.46	2.3	0.01	3.9	<0.0001
Acceleration time (msec)	60.6	1.37	12.4	0.10	19.6	<0.0001
Mitral valve						
Time-averaged velocity (cm/sec)	17.4	0.30	0.8	0.01	1.3	<0.0001
Peak E-wave velocity (cm/sec)	34.4	0.76	5.5	0.08	11.9	<0.0001
Peak A-wave velocity (cm/sec)	47.4	0.17	5.3	0.06	12.9	0.0008
E/A ratio	0.73	0.01	0.001	0.00001	0.005	<0.0001
Tricuspid valve						
Time-averaged velocity (cm/sec)	19.0	0.33	1.05	0.006	1.7	<0.0001
Peak E-wave velocity (cm/sec)	39.6	0.85	4.5	0.09	10.6	<0.0001
Peak A-wave velocity (cm/sec)	54.0	0.31	3.8	0.06	12.6	<0.0001
E/A ratio	0.74	0.013	0.0006	<0.00001	0.002	<0.0001
Pulmonary artery/ascending aorta peak systolic velocity	0.80	0.001	0.0003	0.00002	0.03	1.0
Pulmonary artery/ascending aorta time-averaged velocity	0.91	0.006	0.001	0.00005	0.007	0.0005
Pulmonary artery/ascending aorta acceleration time	0.85	0.014	0.003	0.00003	0.009	<0.0001
Tricuspid valve/mitral valve time-averaged velocity	1.1	−0.0006	0.001	0.000006	0.009	0.9

Sa, Standard error of the intercept; *Sb,* standard error of the slope; *x,* gestational age

Period time in this study ranged from 361 to 535 msec, corresponding to a fetal heart rate of 166 to 112 beats/min, respectively. Average period time showed a significant increase with advancing gestational age, the regression equation being

$$y = 380.2 + 1.33x$$

($p < 0.0001$), where y is average period time (milliseconds), and x is gestational age (weeks).

In those fetuses in which it was not possible to separate E wave and A waves at mitral level, the average time period (± 1 SD) was 393 ± 20 msec, compared with an average of 425 ± 16 msec in fetuses with distinct mitral E and A waves. At tricuspid level these figures were 400 ± 22 and 423 ± 18 msec, respectively.

All parameters studied showed a significant increase with advancing gestational age. Peak systolic and time-averaged velocity, as well as acceleration time, showed higher values in the aorta compared with the pulmonary artery in most fetuses. The ratio for pulmonary to aortic peak systolic velocity did not change during the second half of pregnancy, with a mean ratio of 0.8 ± 0.0003 (1 SD). The ratio for pulmonary to aortic time-averaged velocity, however, increased with advancing gestational age ($p = 0.0005$), as did the ratio for pulmonary to aortic acceleration time ($p = 0.0001$). Time-averaged velocity and peak A-wave and E-wave velocities were higher at tricuspid level than at mitral level ($p < 0.0001$). E/A ratio was higher at mitral level than at tricuspid level ($p < 0.03$). The ratio for tricuspid-to-mitral time-averaged velocity did not change with advancing gestational age, the mean ratio being 1.097 ± 0.001 (1 SD). These results are also summa-

Table IIA. Mean left-to-right differences for ascending aorta and pulmonary artery, with their respective levels of significance

	Ascending aorta	*Pulmonary artery*	*Difference*		*p Value*
			Absolute	*SD*	
Mean peak systolic velocity (cm/sec)	73.0	58.4	14.6	3.3	<0.001
Mean time-averaged velocity (cm/sec)	23.4	21.0	2.4	1.4	<0.001
Mean acceleration time (msec)	58.5	48.5	10.0	4.3	<0.001

The figures are based on the mean paired differences per fetus (paired *t* test).

Table IIB. Mean left-to-right differences for mitral valve and tricuspid valve, with their respective levels of significance

	Mitral valve	*Tricuspid valve*	*Difference*		*p Value*
			Absolute	*SD*	
Mean time-averaged velocity (cm/sec)	16.9	18.5	−1.6	0.9	<0.001
Mean peak E-wave velocity (cm/sec)	33.2	37.2	−4.0	0.4	<0.001
Mean peak A-wave velocity (cm/sec)	47.0	53.6	−6.6	2.3	<0.001
Mean E/A ratio	0.71	0.69	0.02	0.007	<0.03

The figures are based on the mean paired differences per fetus (paired *t* test).

rized in Table I. In Tables IIA and IIB mean absolute differences (±1 SD) between all parameters for the right and left sides of the heart are presented with their corresponding level of significance. At the outflow tract level, no correlation could be demonstrated for any of the parameters and period times. The correlation coefficients at atrioventricular level are presented with their respective *p* values in Table III; negative correlations were found for time-averaged velocity and peak A-wave velocity at both sites. A positive correlation existed between period time and peak E-wave velocity, as well as E/A ratio.

Comment

In the human fetal heart, both right and left ventricles eject in parallel into the systemic circulation: the right ventricle into the ductus arteriosus and descending aorta; the left ventricle into the ascending aorta.[10] Therefore right and left ventricular output are not necessarily equal. In fact, many fetal echocardiographic studies are indicative of a distinctive right-heart dominance in the human fetus,[1, 2] although this is disputed by some.[11]

In this prospective, longitudinal study an increase with advancing gestational age was found for all flow parameters concerned. The distribution of the parameters increased toward term; this is in agreement with the increasing variance of biometric parameters with advancing gestational age. Fetal heart rate showed a significant decline toward term, as has been reported earlier.[4]

Cardiac performance is influenced by heart rate, preload, afterload, and intrinsic properties of both ventricles. From lamb studies it appears that cardiac function is particularly sensitive to changes in afterload,[12] which is determined by blood pressure and vascular resistance. The gestational age–related rise in normal peak systolic flow velocity and time-averaged velocity at semilunar level may be accounted for by increased volume flow through the semilunar valves, raised contractility, or reduced afterload. Age-related reduction in afterload may occur in the human fetus as a result of the physiologic decrease in placental vascular resistance, as expressed by increased end-diastolic flow velocities in the umbilical artery during the second half of gestation.[13] Peak systolic velocity correlates to some extent with time-averaged velocity. Volume flow is equal to mean velocity multiplied by area; therefore the observed increase in peak systolic and time-averaged velocity also may reflect increased left and right ventricular stroke volume and output. The noninvasive nature of human fetal Doppler studies does not allow differentiation between these explanations.

Calculation of volume flow was not attempted, because of the considerable error related to vessel and valve area measurements.[14] Therefore we cannot pro-

Table III. Correlation coefficients and *p* levels for relationship between period time and atrioventricular flow velocity parameters interpolated in a linear fashion to a gestational age of 30 weeks

	r	*p Value*
Mitral valve		
Time-averaged velocity (cm/sec)	−0.34	0.03
Peak E-wave velocity (cm/sec)	0.71	<0.001
Peak A-wave velocity (cm/sec)	−0.32	0.05
E/A ratio	0.67	<0.001
Tricuspid valve		
Time-averaged velocity (cm/sec)	−0.42	0.007
Peak E-wave velocity (cm/sec)	0.55	<0.001
Peak A-wave velocity (cm/sec)	−0.48	0.002
E/A ratio	0.59	<0.001

vide data concerning the presumed right ventricular dominance in the human fetus. The linear increase in peak systolic velocity in both arteries with advancing gestational age is in agreement with other studies, as are the absolute values.[1-4] Also, time-averaged velocity showed a linear increase with advancing gestational age. These data are in agreement with those presented by Kenny et al.[4] but are at variance with other reports in which no relationship between peak systolic[1] and time-averaged velocity[1, 6] and pregnancy duration could be established at semilunar level. The only explanation for these discrepant findings could be the fact that in the present study only flow velocity waveforms obtained at an interrogation angle of ≤10 degrees were collected. This is in contrast to the other reports in which interrogation angles up to 30 degrees were accepted.

The higher peak systolic and time-averaged velocities in the ascending aorta compared with the pulmonary artery observed in normal pregnancy may be due to the difference in semilunar valve area between the two vessels.[1] Alternatively, relatively low fetal cerebral vascular resistance with a subsequently lower left ventricular afterload may be responsible for the documented difference.[15] Of interest is that the acceleration time in the pulmonary artery was shorter than in the aorta, which is in agreement with a previous study by Machado et al.[9] Doppler echocardiographic studies on pulmonary hypertension in adults have demonstrated a close negative correlation between acceleration time and mean arterial pressure in the pulmonary artery as measured by cardiac catheterization.[16, 17] If a similar correlation is assumed in the normally developing fetus, the difference in acceleration time between the two outflow tract vessels would be in support of a different afterload in the two circuits.

In contrast to Machado et al.[9] we found an increase in acceleration time with advancing gestational age for both vessels. This is not surprising, since the afterload to both ventricles is determined mainly by placental vascular resistance, which is characterized by a fall during the second half of the pregnancy.[13] Moreover, both cerebral vascular resistance,[15] which is partly responsible for left ventricular afterload, and pulmonary vascular resistance[18] are subject to a reduction during the third trimester. This may explain the ratio increase with advancing gestational age for both pulmonary to aortic time-averaged velocity and acceleration time.

No correlation could be found between period time and any of the outflow tract parameters. This is in agreement with other observations, which show that only in cases of abnormally high or low fetal heart rates do the effects of the Frank-Starling mechanism become apparent.[19, 20]

Time-averaged velocity at tricuspid and mitral level increased with advancing gestational age with higher tricuspid velocities in nearly all fetuses, reflecting a rising ventricular output and/or decreasing afterload and/or improving myocardial contractility during the second half of pregnancy. Whereas similar results with slightly lower absolute values were reported by Reed et al.[6] and De Smedt et al.,[21] no such increase was established by Allan et al.[1] Peak E-wave and peak A-wave velocities, as well as E/A ratios, displayed a rise at both mitral and tricuspid level, with absolute values of peak A-wave and peak E-wave velocities being higher at tricuspid valve level. E/A ratios were higher at mitral valve level. The increase in peak E-wave velocity and E/A ratio suggests a shift of blood flow from late toward early diastole as a result of increased ventricular compliance with advancing gestational age. Similar observations have been made by others.[4, 6] Improvement in atrial contractility may be the cause for the rising peak A-wave velocities. The higher E/A ratios at mitral level again may reflect a relatively lower left ventricular afterload.

Our findings are partly at variance with those reported by others. Reed et al.[6] observed an increase of tricuspid peak E-wave velocity and a decrease of mitral peak A-wave velocity with advancing gestational age, while tricuspid peak A-wave velocity and mitral peak E-wave velocity did not show a significant change. Also, the absolute values were lower than observed in the present study. Hata et al.[3] reported an increase of both mitral and tricuspid peak A-wave velocity during the second and third trimesters but did not study peak E-wave velocities. At first sight these discrepancies seem quite impressive. The possible influence of the use of angle correction has been mentioned earlier. Moreover, none of these authors has taken into account the influence of period time on atrioventricular waveforms, which could explain, at least in part, the observed differences. In our study a negative correlation between peak A-wave velocity and time-averaged velocity and period time was established within the normal heart rate range at both atrioventricular valve levels. A positive correlation with period time for peak E-wave velocities and E/A ratios was found. This indicates that blood volume shifts from late to early diastole at lower fetal heart rates, thereby resulting in waveforms resembling those documented in older fetuses with more compliant ventricles.

The negative correlation between atrioventricular time-averaged velocity and period time may be explained by changing valve area, as has been suggested by Kenny et al.[22] This could be due to changes in ventricular filling. In conclusion, our results show that the normal second half of pregnancy is characterized by increasing flow velocities at both atrioventricular and outflow tract level, suggesting rising ventricular stroke volume and output and reduction in ventricular afterload. Flow velocity and acceleration time in the ascending aorta suggest a relatively lower afterload to the left ventricle. Transmitral and transtricuspid flow velocities are heart rate dependent.

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Effect of cigarette smoking on maternal airway function during pregnancy

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The effects of cigarette smoking on maternal airway function during pregnancy were investigated in a cross-sectional study of 97 smokers and 175 nonsmokers at different gestational ages. The groups were comparable in age, height, and weight. All subjects were healthy. Forced vital capacity, forced expiratory volume in 1 second, their ratio, the forced expiratory flow rates between 0.2 and 1.2 L, 25% and 75%, and 75% and 85%, and instantaneous flows at lung volumes of 25%, 50%, and 75% were measured. All spirometric tests were unaffected by gestational age. However, all parameters of spirometry were significantly less in smokers than in nonsmokers when cumulative data during pregnancy were compared. Forced vital capacity, forced expiratory volume in 1 second, and their ratio were minimally reduced (4%, $p < 0.05$; 8%, $p < 0.001$; and 4%, $p < 0.001$; respectively) in smokers as compared with nonsmokers. Larger reductions were noted in forced expiratory flow rates between 0.2 and 1.2 L (14%, $p < 0.001$) and between 25% and 75% (16%, $p < 0.001$), and in instantaneous maximum flows at lung volumes of 75% (11%, $p < 0.001$) and 50% (13%, $p < 0.001$). Maximum reduction of forced expiratory flow rates between 75% and 85% (26%, $p < 0.001$) and in instantaneous flows at maximum lung volumes of 25% (23%, $p < 0.001$) suggests marked increases in small-airway resistance and early small-airway disease in smokers. The progression of small-airway disease is related to the level of cigarette exposure. The results of our study demonstrate that the bronchodilatory effect expected in pregnancy is not sufficient to overcome the deleterious effects of cigarette smoking. (Am J Obstet Gynecol 1991;165: 675-9.)

Key words: Cigarette smoke, pregnancy, airway function

It has long been recognized that pregnancy can alter lung function and the natural history of certain pulmonary diseases. Although maximum flow rate and forced expiratory volume in 1 second (FEV_1) are not measurably altered,[1] the reduced total pulmonary resistance and increased airway conductance[2] observed during pregnancy suggest a bronchodilatory effect. This improvement in respiratory function is attributed to an increased concentration of progesterone, which is known to have a marked smooth-muscle–relaxing effect.[3] Indeed, progesterone has been given therapeutically to patients with obesity hypoventilation syndrome,[4] asthma,[5] and chronic obstructive pulmonary disease.[6] It could be hypothesized that the deleterious effects of smoking on airway function may be decreased during pregnancy, especially during the third trimester when progesterone levels are highest. Although cigarette smoking can cause airflow limitation in nonpregnant women,[7] little information is available on its effects on maternal airway function during pregnancy. This study was designed to assess the effects of cigarette smoking on airway function in normal pregnant women and to determine whether these effects are related to gestational age.

Material and methods

This was a cross-sectional study of women in each of the three trimesters of pregnancy who were recruited after written informed consent. The study was performed at the antenatal clinic of Saint-François D'Assise Hospital, a tertiary care teaching hospital associated with Laval University in Quebec City. Included in the study were women with nulliparous or multiparous singleton pregnancies who had no evidence of cardiopulmonary or obstetric disorders. Smokers had to abstain from smoking for ≥2 hours before the test. Subjects were excluded if satisfactory measurements could not be taken because of a lack of cooperation or an inability to perform the tests. Ex-smokers were retrospectively excluded because this group was too small to permit meaningful analysis. The study groups consisted of 97 pregnant smokers and 175 pregnant nonsmokers. Patients were grouped into three categories on the basis of gestational age: Group A (first trimester, 7 to 13 weeks) included 59 nonsmokers and 28 smokers; group B (second trimester, 14 to 26 weeks) included 56 nonsmokers and 35 smokers; group C (third trimes-

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Table I. Anthropometric and cigarette smoking data of pregnant women

	Nonsmokers *(n = 175)*	*Smokers* *(n = 97)*	*p Value*
Age (yr)	28.0 ± 3.52	27.3 ± 4.26	NS
Height (cm)	161.7 ± 5.39	160.8 ± 5.26	NS
Weight (kg)	64.4 ± 11.06	64.2 ± 11.56	NS
Cigarettes/day	—	16.0 ± 6.55	—
Years of smoking	—	10.4 ± 3.76	—

Data are presented as mean ± SD. *NS,* Not significant.

Table II. Forced expiratory spirography in nonsmokers during pregnancy

Variable	*Group A:* *First trimester* *(n = 59)*	*Group B:* *Second trimester* *(n = 56)*	*Group C:* *Third trimester* *(n = 60)*
Age (yr)	27.66 ± 3.22	27.89 ± 3.97	28.48 ± 3.36
Height (cm)	161.33 ± 5.66	162.06 ± 5.47	161.68 ± 5.11
FVC (L)	3.90 ± 0.51	3.89 ± 0.54	4.00 ± 0.45
FEV_1 (L)	3.34 ± 0.42	3.33 ± 0.41	3.41 ± 0.34
$FEV_{1\%}$	85.75 ± 4.09	85.75 ± 4.54	85.47 ± 3.82
$FEF_{0.2\text{-}1.2}$ (L/sec)	6.52 ± 1.27	6.70 ± 1.21	6.87 ± 1.16
$FEF_{25\%\text{-}75\%}$ (L/sec)	3.77 ± 0.71	3.81 ± 0.72	3.90 ± 0.64
$FEF_{75\%\text{-}85\%}$ (L/sec)	1.41 ± 0.36	1.39 ± 0.37	1.37 ± 0.33
Vmax 75% (L/sec)	6.11 ± 0.87	6.13 ± 1.07	6.21 ± 1.24
Vmax 50% (L/sec)	4.31 ± 0.81	4.33 ± 0.97	4.40 ± 0.68
Vmax 25% (L/sec)	1.90 ± 0.44	1.93 ± 0.49	1.92 ± 0.50

Data are presented as mean ± SD. p Value of all parameters between trimesters not significant. *FVC,* Forced vital capacity.

ter, 27 to 40 weeks) included 60 nonsmokers and 34 smokers. Each subject was studied as a member of only one gestational group.

Spirometric tests were conducted with a wedge bellow spirometer (Vitalograph Medical Instruments Ltd., Buckingham, England). Standard test methods were adhered to, with the patient in a standing position as recommended by the American Thoracic Society.[8] The spirometer was calibrated against a known volume of air with a precision syringe.[8, 9] Specific measurements of forced expiratory spirogram included forced vital capacity, FEV_1, FEV_1 expressed as a percentage of forced vital capacity ($FEV_{1\%}$), maximum expiratory flow rate ($FEF_{0.2\text{-}1.2\ L}$), maximum midexpiratory flow rate ($FEF_{25\%\text{-}75\%}$), maximum end-expiratory flow rate ($FEF_{75\%\text{-}85\%}$), and instantaneous flow (Vmax) at 75%, 50%, and 25% of remaining forced vital capacity. The expiratory efforts were maintained in most trials for 6 seconds. The best effort of three values within 5% or 100 ml of each other was used for the analysis. The maximum forced vital capacity and FEV_1 values were recorded, and flow rates were calculated from the curve with the greatest sum of forced vital capacity and FEV_1. All values were corrected for body temperature and pressure saturated with water vapor.

For each of the spirometric tests in both smokers and nonsmokers, the difference between means (±SD) was evaluated with the unpaired Student t test. One-way analysis of variance was also used for comparisons involving more than two groups. The relationship between smoking habit and lung function was assessed with multiple regression analysis. The alpha error for a significant test was set at the 5% level. The percentage of difference between the smoking and nonsmoking groups was assessed for each of the spirometric tests.

Results

The characteristics of the pregnant women are presented in Table I. The groups were comparable for age, height, and weight. Among smokers the daily consumption was 16.0 ± 6.55 (mean ± SD) cigarettes for a duration of 10.4 ± 3.76 (mean ± SD) years.

Table II illustrates the forced expiratory spirogram during the various trimesters for nonsmoking pregnant women. Forced vital capacity, FEV_1, $FEV_{1\%}$, $FEF_{0.2\text{-}1.2\ L}$, $FEF_{25\%\text{-}75\%}$, $FEF_{75\%\text{-}85\%}$, and 75% Vmax, 50% Vmax, and 25% Vmax were not affected by advancing gestational age. This was also the case in the smoking group (Table III).

When cumulative data during pregnancy were compared, the values of all spirometric parameters were significantly less among smokers as compared with nonsmokers (Table IV). More marked differences between groups were noted in both FEF and Vmax than in forced vital capacity, FEV_1, and $FEV_{1\%}$. The greatest differences were observed in $FEF_{75\%\text{-}85\%}$ and 25% Vmax (26% and 23%, respectively; $p < 0.001$).

The dose-response relationship between the level of

Table III. Forced expiratory spirography in smokers during pregnancy

Variable	*Group A: First trimester (n = 28)*	*Group B: Second trimester (n = 35)*	*Group C: Third trimester (n = 34)*
Age (yr)	27.85 ± 4.26	26.71 ± 3.78	27.50 ± 4.75
Height (cm)	161.02 ± 5.49	161.16 ± 4.64	160.34 ± 5.75
Cigarettes/day	16.20 ± 6.69	17.20 ± 6.81	15.10 ± 6.17
Years of smoking	10.90 ± 4.39	10.30 ± 3.60	10.00 ± 3.41
FVC (L)	3.75 ± 0.51	3.88 ± 0.52	3.72 ± 0.62
FEV_1 (L)	3.04 ± 0.45	3.17 ± 0.46	3.07 ± 0.45
$FEV_{1\%}$	81.04 ± 7.09	81.82 ± 6.32	83.27 ± 5.32
$FEF_{0.2\text{-}1.2\text{ L}}$ (L/sec)	5.60 ± 1.44	6.05 ± 1.42	5.59 ± 0.89
$FEF_{25\%\text{-}75\%}$ (L/sec)	3.06 ± 0.75	3.22 ± 0.84	3.33 ± 0.68
$FEF_{75\%\text{-}85\%}$ (L/sec)	0.98 ± 0.32	1.07 ± 0.40	1.01 ± 0.32
Vmax 75% (L/sec)	5.35 ± 1.34	5.69 ± 1.28	5.26 ± 0.81
Vmax 50% (L/sec)	3.60 ± 0.97	3.70 ± 0.92	3.94 ± 0.79
Vmax 25% (L/sec)	1.48 ± 0.56	1.46 ± 0.48	1.48 ± 0.46

Data are presented as mean ± SD. *p* Value of all parameters between trimesters not significant.

Table IV. Forced expiratory spirography during pregnancy

Variable	*Nonsmokers (n = 175)*	*Smokers (n = 97)*	*Difference (%)*	*p Value*
FVC (L)	3.93 ± 0.49	3.78 ± 0.55	−4	<0.05
FEV_1 (L)	3.36 ± 0.39	3.09 ± 0.45	−8	<0.001
$FEV_{1\%}$	85.65 ± 4.13	82.10 ± 6.23	−4	<0.001
$FEF_{0.2\text{-}1.2\text{ L}}$ (L/sec)	6.70 ± 1.22	5.76 ± 1.27	−14	<0.001
$FEF_{25\%\text{-}75\%}$ (L/sec)	3.85 ± 0.69	3.21 ± 0.76	−16	<0.001
$FEF_{75\%\text{-}85\%}$ (L/sec)	1.39 ± 0.35	1.03 ± 0.35	−26	<0.001
Vmax 75% (L/sec)	6.15 ± 1.07	5.44 ± 1.16	−11	<0.001
Vmax 50% (L/sec)	4.35 ± 0.82	3.76 ± 0.89	−13	<0.001
Vmax 25% (L/sec)	1.91 ± 0.47	1.47 ± 0.49	−23	<0.001

Data are presented as mean ± SD. *FVC,* Forced vital capacity.

cigarette consumption and lung function is shown in Table V. The number of cigarettes smoked per day was directly related to the difference between the groups. This relationship was also confirmed by multiple regression analysis when adjustments were made for confounding variables such as age, height, and duration of smoking.

Comment

Airway function during pregnancy could be influenced by anatomic, hormonal, or biochemical factors. Progressive enlargement of the gravid uterus, mainly in the third trimester, may produce a restrictive effect. The reported reduction in alveolar Pco_2 in pregnancy, which is responsible for bronchial smooth-muscle constriction, can cause obstruction in airway function by increasing airway resistance.[10, 11] Our observation in both the nonsmoking and smoking groups showed no significant alteration in forced vital capacity, FEV_1, FEF, and Vmax at different lung volumes as pregnancy advanced; therefore there may be other mechanisms operating in pregnancy to oppose the expected alteration of airway function. Alternatively, those factors may have insufficient influence to modify the respiratory function as measured.

The results concerning forced vital capacity, FEV_1, and $FEV_{1\%}$ during the three trimesters in nonsmoking pregnant women are in agreement with previous studies in which serial measurements were performed.[12, 13] FEF and Vmax at different lung volumes have not been fully studied during pregnancy. In two studies,[1, 14] $FEF_{25\%\text{-}75\%}$, 50% Vmax, and 25% Vmax were measured and found to be unaltered during pregnancy; there were similar findings in our study. $FEF_{0.2\text{-}1.2\text{ L}}$, $FEF_{75\%\text{-}85\%}$, and 75% Vmax were also unchanged. There was no relationship between any of the studied parameters and gestational age. Preservation of airway function in the second half of pregnancy in spite of the enlarged gravid uterus may be caused by bronchodilating effects. Progesterone is known to have a widespread smooth-muscle–relaxing effect[3] and an increased β-adrenergic activity.[15] The relative mobility of the thoracic cage[16] may also be responsible for preserving forced vital capacity.

This is the first report of a significant decrease in airway function among pregnant smokers as compared with pregnant nonsmokers. Maximum reductions were seen in tests reflecting small-airway function. The decrease in forced vital capacity (4%, $p < 0.05$) could be explained by a narrowing or closure of the small air-

Table V. Effect of amount of cigarette smoking on forced expiratory spirography in pregnancy

	FVC (L)	*FEV$_1$ (L)*	*FEF$_{0.2\text{-}1.2\ L}$ (L/sec)*	*FEF$_{25\%\text{-}75\%}$ (L/sec)*	*FEF$_{75\%\text{-}85\%}$ (L/sec)*	*Vmax 75% (L/sec)*	*Vmax 50% (L/sec)*	*Vmax 25% (L/sec)*
Group 1: Nonsmokers (*n* = 77)	3.88 ± 0.48	33.3 ± 0.39	6.78 ± 1.26	3.87 ± 0.73	1.43 ± 0.36	6.13 ± 1.18	4.36 ± 0.88	1.99 ± 0.49
Group 2: Light smokers* (*n* = 59)	3.76 ± 0.51	3.15 ± 0.37	5.91 ± 0.98	3.45 ± 0.63	1.11 ± 0.32	5.56 ± 0.92	4.06 ± 0.73	1.57 ± 0.42
Group 3: Heavy smokers† (*n* = 38)	3.82 ± 0.61	3.01 ± 0.55	5.53 ± 1.62	2.85 ± 0.81	0.89 ± 0.36	5.25 ± 1.46	3.28 ± 0.92	1.33 ± 0.57
Group 1 vs 2	$p < 0.05$	$p < 0.001$	NS	NS	$p < 0.01$	$p < 0.05$	$p < 0.001$	$p < 0.05$
Group 1 vs 3	$p < 0.001$	$p < 0.001$	NS	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Group 2 vs 3	$p < 0.001$	$p < 0.05$	NS	NS	NS	$p < 0.001$	$p < 0.01$	NS

Data are presented as mean ± SD. *FVC,* Forced vital capacity; *NS* = not significant.

*1 to 15 cigarettes per day for 10.3 years.

†16 to 30 cigarettes per day for 10.5 years.

ways, because 20% of forced vital capacity is thought to be related to these structures.[17] Loss of the elastic recoil pressure of the lung may also cause forced vital capacity to fall.

The reductions in FEV$_1$ (8%, $p < 0.001$) and FEV$_{1\%}$ (4%, $p < 0.001$) seen among smokers suggest an overall airway obstruction, although neither bronchoconstriction, compliance, nor recoil was actually measured. Because FEV$_1$ is a measure of flow rate during the first second of forced expiratory spirography, it includes sufficient flows at lower lung volumes to reflect small-airway changes in addition to large-airway changes.

The decreased flow at large lung volumes, such as FEF$_{0.2\text{-}1.2\ L}$ (14%, $p < 0.001$) and 75% Vmax (11%, $p < 0.001$) may be caused by either an increase in large airway resistance or a decrease in effort, inasmuch as these flows are largely effort dependent. Although subjects performed tests using maximum effort, a decrease in the expiratory muscle power among smokers cannot be entirely ruled out.

In contrast, FEF$_{25\%\text{-}75\%}$ and FEF$_{75\%\text{-}85\%}$ are independent of effort and are important to detect small-airway patency, particularly an airway with a diameter <2 mm. In smokers the contribution of small-airway resistance to total-airway resistance normally increases at low lung volumes, even when FEV$_1$ is not clinically abnormal. In the smoking group there was marked deterioration in both FEF$_{25\%\text{-}75\%}$ (16%, $p < 0.001$) and FEF$_{75\%\text{-}85\%}$ (26%, $p < 0.001$). A 25% reduction in FEF$_{75\%\text{-}85\%}$ is considered to be clinically abnormal[18]; however, the sensitivity of FEF$_{25\%\text{-}75\%}$ to dysfunction is not necessarily greater than that of FEV$_1$ because the FEF$_{25\%\text{-}75\%}$ has higher within- and between-individual variability.[19]

Although direct airway resistance and elastic recoil pressure or lung compliance were not measured, it is assumed that the observed decrease in flow rates at low lung volumes is caused by increased resistance to flow rather than a decrease in driving force. Alteration in lung elastic recoil after smoking cannot be entirely ruled out; however, in normal pregnant women lung elastic recoil should not be altered because lung compliance has been shown to remain unchanged during pregnancy compared with the postpartum period.[2]

The measurement of Vmax showed significant reductions in smokers. The maximum percentage of diffrence between smokers and nonsmokers was seen in 25% Vmax, suggesting early small-airway disease in smokers even though the subjects were asymptomatic. The relationship between the progression of small-airway dysfunction and smoking habits is confirmed by a direct relationship between the level of cigarette exposure and the degree of lung function loss. Further, there is a nicotine-induced increase in oxygen consumption with a reduced oxygen supply caused by carbon monoxide in smokers.[20] These factors could lead to a reduced supply of oxygen to the fetus. This reduced oxygen supply could contribute to intrauterine growth retardation.

In summary, changes in airway function in smokers during pregnancy were similar to those observed in nonpregnant smokers.[7] It can be concluded that the bronchodilatory effect expected in pregnancy is not sufficient to overcome the deleterious effect of cigarette smoking.

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Effectiveness of antibiotic prophylaxis in preventing bacteriuria after multichannel urodynamic investigations: A blind, randomized study in 124 female patients

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One hundred twenty-four women with chronic, persistent lower urinary tract symptoms who had been scheduled for elective urodynamic investigations at Mount Sinai's Urodynamic Investigative Unit were divided into two blind, randomized groups, receiving either a placebo or prophylactic antibiotic. At the time of urodynamic testing, the rate of unsuspected urinary tract infection was 8.1%. There was no statistically significant decrease in postinstrumentation infection rate in the group who received prophylactic antibiotics. We conclude that, given in the fashion described in the study, prophylactic antibiotics are not effective in preventing urinary tract infections caused by urodynamic testing. (AM J OBSTET GYNECOL 1991;165:679-81.)

Key words: Prospective, randomized, prophylactic antibiotics, complex multichannel urodynamic investigation

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Recent cases of bacteriuria after urodynamic investigations performed in the urogynecology unit at Mount Sinai Hospital prompted us to review the current literature and to question whether routine prophylactic antibiotics should be given after urodynamic testing. Previously, antibiotic prophylaxis was not routine in our investigative laboratory. Previous reports

Table I. Patient demographics

	Group A	*Group B*	*p Value*
No. of patients	49	53	
Age (yr)			
Mean	55.29	58.58	NS
Range	27-77	24-81	
Gravidity			
Mean	3.04	3.09	NS
Range	0-10	0-8	
Parity			
Mean	2.43	2.58	NS
Range	0-8	0-7	
Weight (kg)			
Mean	69.89	69.78	NS
Range	49-98	50-106	
Patients with infections on follow-up			
No.	4	10	NS
%	8.2	18.9	

NS, Not significant.

have shown one-time bladder catheterization to result in a 2% incidence of urinary tract infection.[1] Bergman and McCarthy[2] reported a 4% incidence of acquired infection in a control group, compared with 0% in a treatment group. They concluded that this difference was not statistically or clinically significant because the numbers in each of the groups were small. They found a 7.3% incidence of unsuspected infection at the time of urodynamic evaluation; they suggested that a short course of prophylactic antibiotics before testing would be in order.

Similarly, Coptcoat et al.[3] found positive cultures in 2 (10%) of 20 women in a control group, compared with 0 (0%) of 18 women in a prophylactic antibiotic group. They concluded that antibiotic prophylaxis was not worthwhile in patients with normal upper urinary tracts undergoing cystometrography. However, Payne et al.[4] found that postinstrumentation bacteriuria developed in 10 (15%) of 66 women. This is not an insignificant number.

This prospective study was undertaken to assess the incidence of unsuspected bacteriuria before complete multichannel urodynamic testing and to assess whether antibiotic prophylaxis was effective in preventing significant bacteriuria after urodynamic investigations.

Material and methods

One hundred twenty-seven female patients with chronic, persistent lower urinary tract symptoms were asked to participate in the study during routine visits to the Mount Sinai Hospital's Urodynamic Investigative Unit. All patients had negative urine cultures before evaluation. Patients who were performing intermittent self-catheterization, were allergic to nitrofurantoin, or were receiving antibiotics were excluded from the study, leaving 124 women. Uroflowmetry and perianal electromyographic measurements with surface electrodes were performed with a DISA 2100 uroflow system (DISA Elektronic A/S, DK-2740, Skovlunde, Denmark). Urine specimens were obtained via catheterization immediately after uroflowmetry with a standard 14F catheter using sterile technique. The perineum was prepared with chlorhexidine (Hibitane gluconate 1:2000), followed by medium-flow water cystometry. The DISA 2100 urosystem was used to profile static and dynamic urethral pressure. Each patient was profiled in supine and standing positions; the 22K62 dual-pressure sensor microtip catheter with lumen was used in the bladder and urethra, and the 22K60 single-pressure microtip transducer was used in the vagina.

On completion of the procedures, the patients were randomly assigned to prophylaxis or nonprophylaxis groups according to hospital number. Both the physician and the nurse technician were blind as to which assignment the patient received. Patients in group A received nitrofurantoin 50 mg four times and phenazopyridine hydrochloride 200 mg three times for 1 day. Patients in group B received phenazopyridine hydrochloride only. The code was broken at the completion of the study. A clean-catch urine specimen was obtained for culture and sensitivity testing 2 to 3 days after urodynamic testing.

For the purposes of this study, infection on initial catheter-specimen urine is defined as growth $>10^6$ colony-forming units per liter. For the follow-up clean-catch midstream urine, infection is defined as $>10^8$ colony-forming units per liter.

Methods, definitions, and units conform to the standard recommended by the International Continence Society,[5] except where specifically noted. Statistical analysis consisted of Student's t test (two-tailed) or χ^2 (with Yates' correction for continuity) where applicable.

Results

A total of 124 women were recruited for the prophylactic antibiotic trial. All had recently provided sterile midstream urine specimens before recruitment. Of the 124, 22 did not complete the study: 12 women did not provide posttreatment urine specimens, 8 had initial catheter specimens that tested positive for urinary tract infections, and 2 fell into both categories.

Ten (8.1%) of the 124 women had catheter-specimen urine that tested positive for infection before urodynamic testing, thus giving an unsuspected urinary tract infection rate of 8.1%.

The remaining 102 women were examined for differences in infection rate. Table I lists the demographics of both groups; there were no statistically significant differences between them. Of greater significance was that, with this protocol, the postinstrumentation infection rate was not significantly different whether or not the patient received prophylactic antibiotics.

Comment

Our study and studies previously reported[2, 3] show that the use of prophylactic antibiotics at the time of complex multichannel urodynamic testing does not decrease the postinstrumentation infection rate.

Our 8.1% unsuspected infection rate is similar to that reported by Bergman and McCarthy.[2]

Postinstrumentation infection developed in 10 (18.9%) of the 53 women in the control group, compared with 4 (8.2%) of 49 women who received prophylaxis. Although the 18.9% incidence of postinstrumentation infection is not statistically significant, it is somewhat higher than the previously reported rates of 4%,[2] 10%,[3] and 15%[4] and therefore is clinically important. This higher rate may be attributable to multiple insertions of the catheter during urodynamic study, which increase the chance of creating infection.

The absence of a statistical difference may be because of the relatively small number of patients. Other factors that may affect the results are the dose, duration, and type of prophylactic antibiotic used. Further study of these parameters will be necessary.

There is a high incidence of unsuspected infection before testing and a high incidence of infection after urodynamic testing. To decrease these high rates, perhaps patients should receive prophylactic antibiotic treatment before testing,[2] at least until a study is performed with larger numbers and different antibiotic regimens.

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Genetic identification of pregnancies responsible for choriocarcinomas after multiple pregnancies by restriction fragment length polymorphism analysis

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The pregnancies responsible for two cases of choriocarcinomas were determined by the restriction fragment length polymorphism of human leukocyte antigen–DQ genes in the tumors and leukocytes from the patients, their husbands, and their children. The first case was preceded by three normal pregnancies, and the second case was preceded by two normal pregnancies and a complete hydatidiform mole. Comparison of the restriction fragment length polymorphism patterns revealed that the first case of choriocarcinoma carried the parental genome and originated from the third normal pregnancy, whereas the second case was of androgenetic origin from the complete mole. By choosing an appropriate probe (or probes), restriction fragment length polymorphism analysis is shown to be a useful method for identification of the pregnancy responsible for choriocarcinoma. (AM J OBSTET GYNECOL 1991;165:682-8.)

Key words: Choriocarcinoma, responsible pregnancy, restriction fragment length polymorphism

Choriocarcinoma is a malignant tumor originating from trophoblasts in complete hydatidiform moles, normal, and any other types of pregnancies. If a patient with choriocarcinoma has had multiple antecedent pregnancies, the choriocarcinoma is thought to develop from the last pregnancy on the basis of the interpretation of clinical sequence of these patients. However, the possibility that an earlier pregnancy causes the choriocarcinoma cannot definitely be ruled out. In the case of interrupted postmolar choriocarcinoma, the choriocarcinoma has been proposed to be a legacy from the previous hydatidiform mole rather than from the current nonmolar pregnancy on the basis of the susceptibility of hydatidiform moles to develop choriocarcinoma.[1] Although such proposals remain untested, the accurate identification of the responsible pregnancy is important as a first step toward elucidating the oncogenesis and development of choriocarcinomas.

Genetic studies would determine the responsible pregnancy by proving its identity to choriocarcinoma. The androgenetic origin of complete moles has been demonstrated by chromosomal heteropolymorphism,[2, 3] human leukocyte antigen (HLA) haplotypes,[4, 5] and enzyme polymorphism.[6, 7] In contrast with complete moles, choriocarcinomas often show chromosomal gains, losses, and other abnormalities,[8] and scarcely express HLA antigens[9]; thus further analyses on their chromosomes and HLA haplotypes are difficult. In addition, these genetic markers provide only limited information about a few sites on given chromosomes.

In recent years, many deoxyribonucleic acid (DNA) polymorphisms, which exhibit different lengths of DNA fragments after digestions with certain restriction enzymes (restriction fragment length polymorphisms), have been found in the human genome.[10] On restriction fragment length polymorphism analysis, a highy specific set of alleles at polymorphic loci can identify unrelated persons and also children in a family, except monozygotic twins. Restriction fragment length polymorphism analysis therefore is expected to be a useful and widely applicable method to identify the pregnancy responsible for choriocarcinoma.

By restriction fragment length polymorphism analyses, we determined the responsible pregnancies in two patients with choriocarcinoma preceded by multiple pregnancies; choriocarcinoma developed in one patient after three normal pregnancies, and after two normal pregnancies and a subsequent complete mole in the other patient. Here we show that both choriocarcinomas developed from the last antecedent pregnancies—one from the third normal pregnancy and the other from the complete mole.

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Material and methods

Patients. The first patient had two normal deliveries at the age of 27 and 29 years, but no history of molar pregnancies or abortions. At 31 years old she was delivered of her third child, who weighed 2050 gm at 38 weeks' gestation. Six weeks after the third delivery, she had an emergent hysterectomy indicated by massive uterine bleeding. The tumor mass found in the uterus was histologically proved to be choriocarcinoma. The child is developing normally without elevation of serum human chorionic gonadotropin.

The second patient had two normal deliveries, the first at age 28 years and the second at age 29. The third pregnancy at age 35 was a complete hydatidiform mole. Seven months after termination of the mole, roentgenogram examination showed a metastatic shadow in the chest, accompanied by an elevated level of human chorionic gonadotropin. A hysterectomy was performed and histologic examination confirmed the diagnosis of choriocarcinoma. A part of the surgical specimen was transplanted into nude mice and subsequently produced a developing tumor that was histologically also determined to be choriocarcinoma.

Probes. The restriction fragment length polymorphism probes used in this study were inserts from pDQβ155,[11] an HLA-DQβ cDNA clone; pDQα107,[11] an HLA-DQα cDNA clone; OS-4,[12] a clone containing a sequence assigned to chromosome 18; p4.1H2,[13] a clone containing a sequence assigned to chromosome 19; and pHY10,[14] a clone containing a Y chromosome–specific repeated sequence. These clones were obtained from the Japanese Cancer Research Resources Bank. An insert from a cDNA clone of Leu-2 (CD8),[15, 16] which is a nonpolymorphic single copy gene, was used to standardize the amount of loaded DNA on each lane.

Southern hybridization. DNA was prepared from choriocarcinoma tissue and peripheral blood leukocytes by standard techniques. Each of the DNA samples (7.5 to 10 μg) was digested with an appropriate restriction enzyme, and blotted onto a nylon membrane. The membrane was incubated in prehybridization solution [6× saline solution–sodium phosphate–ethylenediaminetetraacetate (SSPE) buffer, 0.5% sodium dodecyl sulfate (SDS), 2× Denhardt's solution, and 100 μg/ml denatured salmon sperm deoxyribonucleic acid (DNA)] for 2 hours and hybridized with the probes for 16 hours, which had been labeled with phosphorus 32 with a random primer labeling system and mixed at a concentration of 1×10^6 cpm/ml to the prehybridization solution. The membrane was then washed in 2× SSPE, 0.1% SDS, and in 0.2× SSPE, 0.1% SDS for 15 minutes twice each. All procedures were carried out at 65° C. Autoradiography was performed with Kodak XAR-5 film for 1 to 7 days at −70° C with an intensifying screen.

Densitometric analysis. The HLA-DQ restriction fragment length polymorphism bands on the autoradiographs were scanned with a densitometer, and their relative area values were measured with an integrater. The measured band intensity was corrected according to the corresponding intensity of the nonpolymorphic Leu-2 band, which was revealed by the rehybridization of the same membrane with a Leu-2 probe.

Results

Case 1. Genomic DNA isolated from peripheral leukocytes of the patient and the members of her family were digested with PstI, and hybridized with a cDNA probe for an HLA-DQβ chain (chromosome 6). These DNA samples offered several bands, among which four polymorphic bands were numbered from 1 through 4 in order of size (Fig. 1, *A*). The patient had bands 1 and 4, but the husband did not have either of them. All children had one of the bands; thus this segregation pattern indicates that these two bands were independently derived from either of the patient's chromosomes 6. Alternatively, the husband had bands 2 and 3. The first child had both bands, whereas the other two children had only band 3, indicating that band 2 was derived from one of the husband's chromosomes 6, but band 3 from both. These data define the four parental haplotypes: we called them a and b for the patient, and c and d for the husband. Accordingly, the first child had a/c haplotype, the second child had a/d, and the third child had b/d (Fig. 1, *B*).

Genomic DNA simultaneously prepared from the choriocarcinoma tissue offered bands 1, 3 and 4 (Fig. 1, *A*), indicating the three haplotypes a, b, and d. This finding suggests that this tissue contains not only choriocarcinoma cells themselves, but also patient-derived stromal or blood cells. These inevitable components were microscopically observed in the tissue section (data not shown). The absence of band 2 in the tissue excludes the possibility that the choriocarcinoma was derived from the first pregnancy. The possibility of originating from the second or third pregnancies, however, could not be readily determined by the band pattern alone because these later pregnancies showed indistinguishable haplotypes (a/d and b/d) from the choriocarcinoma tissue (a/b/d). To distinguish between them, we measured the intensity of restriction fragment length polymorphism bands with a densitometer.

If the choriocarcinoma had developed from the third pregnancy, band 4 in the tissue would have come from the choriocarcinoma cells and patient-derived cells. The expected intensity value of band 4 of the tissue can be calculated from the measured intensity value of the third pregnancy-derived (but not patient-derived) band 3, and that of the patient-derived band 1 as follows: $10.9 \times (10.0/14.4) + 8.3 \times (3.1/11.1) = 9.8$. This expected value of 9.8 was close to the measured value of 10.1 (Table I). Alternatively, a calculation similar to this excluded the possibility of the second

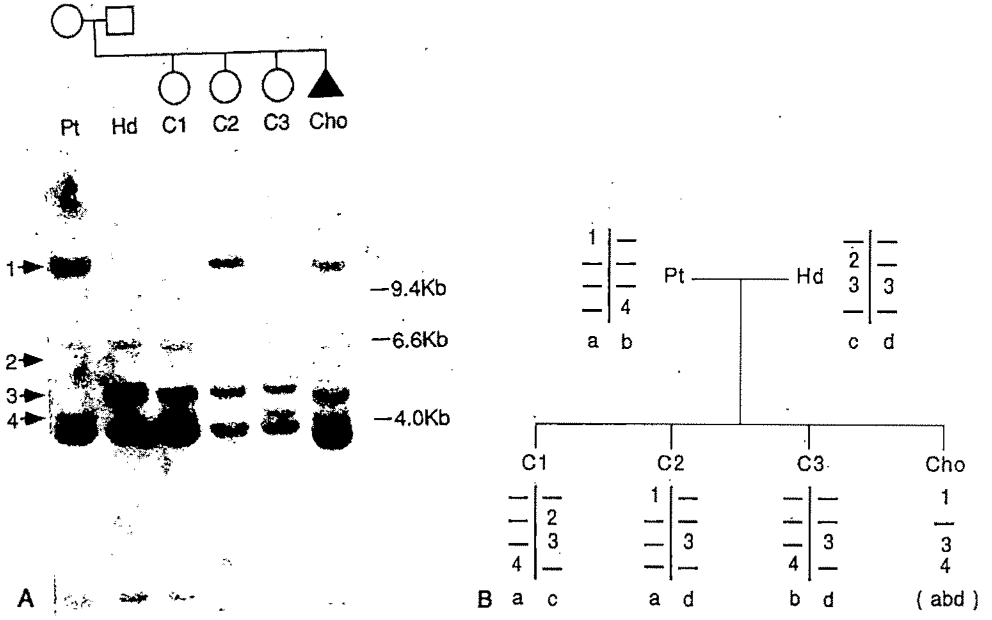

Fig. 1. A, Restriction fragment length polymorphism patterns detected in case 1 by hybridization of *Pst* I–digested DNAs from patient *(Pt)*, husband *(Hd)*, first child *(C1)*, second child *(C2)*, third child *(C3)*, and choriocarcinoma tissue *(Cho)* with HLA-DQβ probe. Each DNA sample shows unique band pattern. Four informative bands are numbered *1* through *4*, as shown on left of lanes. **B,** Segregation pattern of four parental haplotypes in family of case 1. Numbers *1* through *4* show presence of corresponding bands of **A,** and *hyphen* shows absence. Two maternal haplotypes are labeled *a* and *b*, and the two paternal haplotypes are labeled *c* and *d*.

Table I. Densitometric analysis of HLA-DQβ restriction fragment length polymorphism bands in case 1

Band No.	*Densitometric analysis of 3rd pregnancy*			*Densitometric analysis of 2nd pregnancy*		
	3rd Child	*Patient*	*Choriocarcinoma tissue (3rd child + patient)*	*2nd Child*	*Patient*	*Choriocarcinoma tissue (2nd child + patient)*
1	—	11.1	3.1	8.9	11.1	3.1 (19.5)*
3	14.4	—	10.0	14.8	—	10.0
4	10.9	8.3	10.1 (9.8)†	—	8.3	10.1

*The expected intensity value in the case of the choriocarcinoma being developed from the second pregnancy with participation of the patient's stromal cells. The calculation is 8.9 × (10.0/14.8) + 11.1 × (10.1/8.3) = 19.5.

†The expected intensity value in the case of the choriocarcinoma being developed from the third pregnancy with the participation of the patient's stromal cells. The calculation is 10.9 × (10.0/14.4) + 8.3 × (3.1/11.1) = 9.8.

pregnancy origin in which band 1 in the tissue came from the tumor cells themselves and the patient's cells: 8.9 × (10.0/14.8) + 11.1 × (10.1/8.3) = 19.5. But the corresponding measured value of 3.1 was much lower. Taken together, these results indicate that this choriocarcinoma developed from the third pregnancy, not from either of the earlier pregnancies.

Three other theoretically possible origins, although less likely, need to be considered, including unnoticed early abortions of complete moles, nonmolar pregnancies, and germ cells (a nongestational origin). The first possibility of complete mole origin can be ruled out by a comparison of the restriction fragment length polymorphism band intensities. In this case, the maternal element in the choriocarcinoma tissue is derived only from the patient's stromal or blood cells and thus the intensity ratio of maternal band 1 versus band 4 should be identical between the patient and choriocarcinoma tissue. However, this ratio was 3.1/10.1 = 0.3 in the choriocarcinoma tissue sample, whereas it was 11.1/8.3 = 1.3 in the patient sample (Table I). As for the second possibility of nonmolar origin, if the pregnancy had the same haplotype for chromosome 6 as the third child, then it certainly could not be ruled out, and additional restriction fragment length polymorphism analyses on different chromosomes may be re-

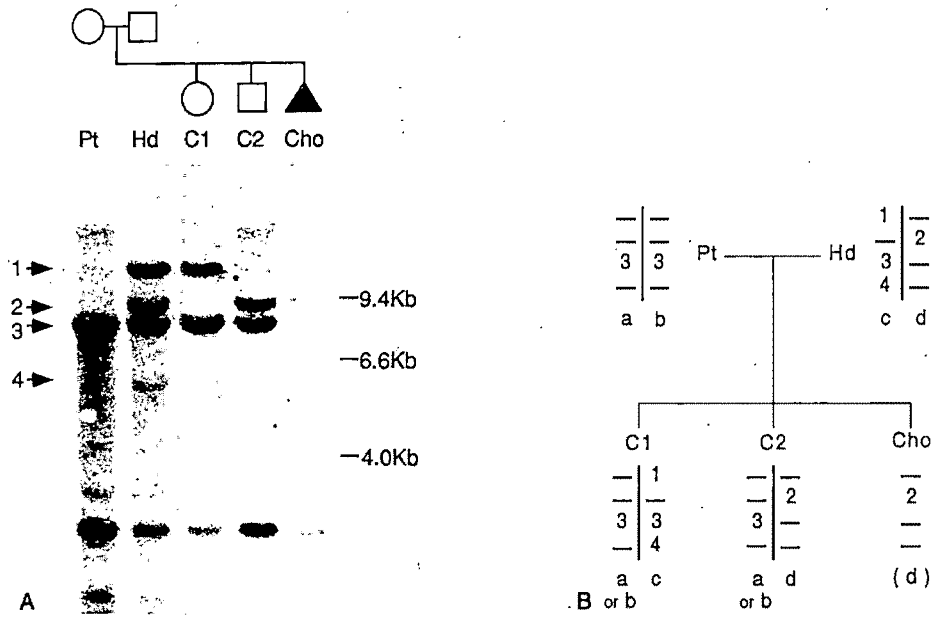

Fig. 2. A, Restriction fragment length polymorphism patterns detected in case 2 by hybridization of *Pst* I–digested DNAs from patient *(Pt)*, husband *(Hd)*, first child *(C1)*, second child *(C2)*, and choriocarcinoma tissue *(Cho)* with HLA-DQα probe. Four informative bands are numbered *1* through *4*, as shown on left of lanes. **B,** Segregation pattern of four parental haplotypes in family of case 2. Numbers *1* through *4* show presence of corresponding bands of **A**, and *hyphen* shows absence. Two maternal haplotypes are labeled *a* and *b*, and two parental haplotypes are labeled *c* and *d*.

quired for verification. The third possibility of a nongestational origin, which is not a result of any paternal genome, was readily excluded by the presence of the paternally derived band 3 in the choriocarcinoma tissue (Fig. 1, *A*).

Case 2. Genomic DNAs from leukocytes of the patient and the members of her family were digested with *Pst* I and hybridized with a cDNA for an HLA-DQα chain. Four polymorphic bands were numbered from 1 through 4 in order of size (Fig. 2, *A*). These four bands were not present in a liver DNA sample of a nude mouse (data not shown). Only the husband and children had bands 1, 2, and 4 in a segregated pattern, in which the husband had all three bands, the first child had bands 1 and 4, and the second child had band 2. This pattern indicates that bands 1 and 4, and band 2 were derived independently from either of the husband's chromosomes 6. Band 3 was commonly detected in this family, but the intensities of these bands measured with densitometry in the patient and the first child were approximately twice as high as those in the husband and the second child (data not shown). This indicates that band 3 in the patient was derived from both chromosomes 6, but that in the husband was from one, which simultaneously contributed to bands 1 and 4. Thus we could define the parental haplotypes a, b, c, and d (Fig. 2, *B*).

Genomic DNA from the choriocarcinoma tissue that was resected from the patient and subsequently transplanted into nude mice had only band 2, apparently differing from the children. The absence of band 3 in the tissue excludes the possibility of contribution of the patient's genome and cells. Although we could not directly analyze the molar DNA, this choriocarcinoma is most likely to have developed from the complete mole. It should be stressed that the presence of only one husband's haplotype, band 2, in the choriocarcinoma indicates its homozygosity for the chromosome 6.

The homozygosity of this choriocarcinoma was further analyzed with several probes showing restriction fragment length polymorphisms on other chromosomes. The DNA samples were digested with *Taq* I and hybridized with an OS-4 probe for a single-copy gene on chromosome 18 (Fig. 3, *A*). The husband had 8.6 and 6.7 kb bands, whereas the choriocarcinoma had only the 8.6 kb band that was absent in the patient. This result indicates that the choriocarcinoma was androgenetic and also homozygous for chromosome 18.

Similarly, the hybridization of *Sma* I digest with a p4.1H2 probe, which is assigned to chromosome 19, revealed that the husband had 9.0 and 8.0 kb bands, whereas the choriocarcinoma lacked the 9.0 kb band (Fig. 3, *B*). In addition, the hybridization of *Eco*RI digest with a Y-chromosome–specific pHY10 probe resulted in a 3.4 kb band in the husband and second male child, but not in the choriocarcinoma (Fig. 3, *C*). These data are compatible with the homozygosity of this cho-

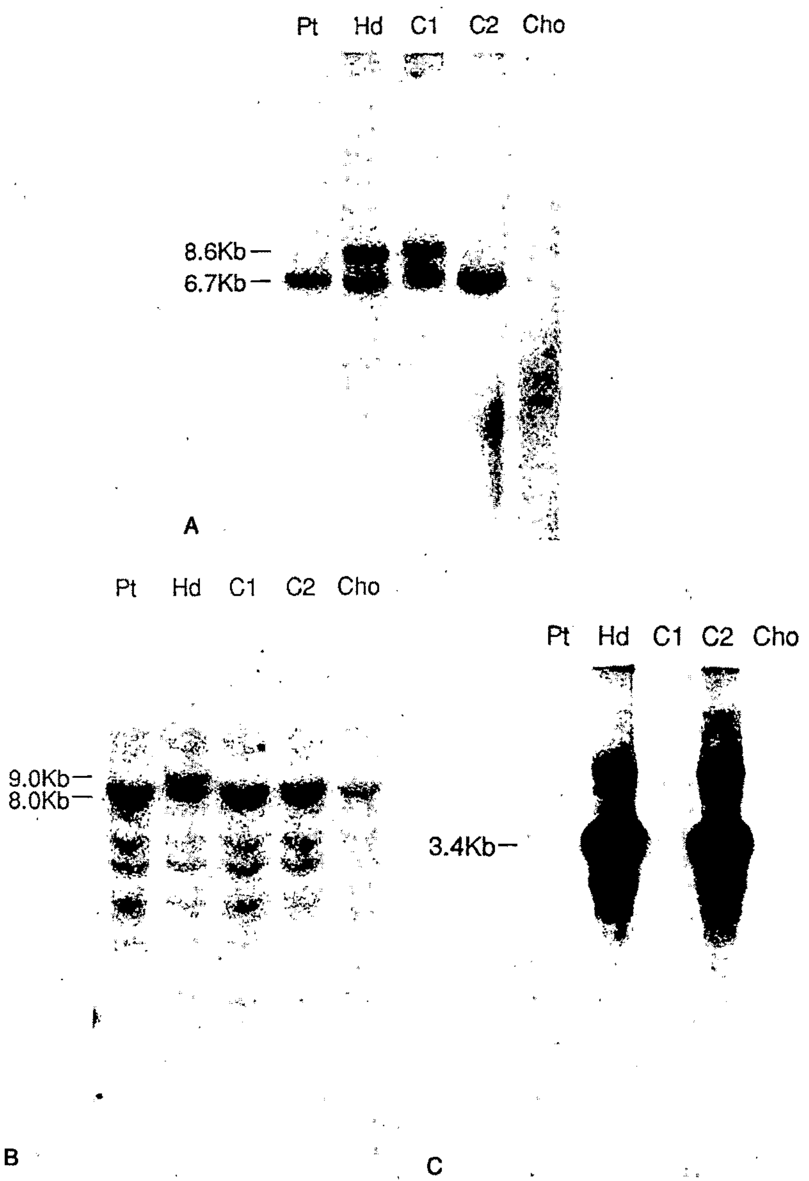

Fig. 3. Restriction fragment length polymorphism patterns detected in case 2 by hybridization of DNAs from patient *(Pt)*, husband *(Hd)*, first child *(C1)*, second child *(C2)*, and choriocarcinoma tissue *(Cho)* with OS-4 **(A)** and p4.1H2 probe **(B)**. Androgenetic origin of choriocarcinoma is shown by restriction fragment length polymorphisms detected with OS-4 probe, whereas homozygous origin is shown by restriction fragment length polymorphisms detected with both OS-4 and p4.1H2 probes. **C,** Southern hybridization of *Eco*RI-digested DNAs from family members in case 2 with Y-chromosome–specific pHY10 probe. Husband and second male child samples show hybridization bands.

riocarcinoma for chromosome 19 and sex because of its androgenetic origin.

No bands were detectable in a nude mouse DNA sample by the hybridization with any of these three probes (data not shown).

Comment

In the first case, a short interval between the third pregnancy and the occurrence of choriocarcinoma empirically suggested that the choriocarcinoma originated from the last pregnancy. Origin from earlier pregnancies, however, remained possible because choriocarcinoma occasionally develops after an extremely long latent interval (e.g., over 10 years, from an antecedent pregnancy).[17] Our restriction fragment length polymorphism analysis, however, directly proved the former assumption. Choriocarcinoma tissue is especially abundant in vascular tracts and blood cells; thus it is likely that this freshly resected choriocarcinoma tissue was contaminated with certain cells of the patient. Densitometric measurements along with careful data analysis could precisely resolve these complicated band patterns from patient-derived cells in our preparation.

In the second case, restriction fragment length polymorphism analysis with HLA-DQα and OS-4 probes showed that the choriocarcinoma was of androgenetic origin. This result indicates therefore that the choriocarcinoma most likely developed from the last complete mole. Because the risk of choriocarcinomas associated with complete moles has been estimated to be between 2000 and 4000 times greater than that of choriocarcinoma with nonmolar pregnancies,[1] the normal pregnancies in this patient were less likely to cause the choriocarcinoma. In this study, we excluded the latter possibility.

A surprisingly high incidence of heterozygosity has been reported in choriocarcinoma arising from postmolar patients by cytogenetic analysis.[18-20] Those results require fundamental reconsideration regarding the direct oncogenic relationship between the choriocarcinoma and complete moles, which mainly (more than 90%) consist of homozygous types. Fisher et al.[21] have reported restriction fragment length polymorphism analysis on two cases of postmolar choriocarcinoma in which one was heterozygous (dispermy) and the other was thought to be homozygous (monospermy). Our androgenetic choriocarcinoma showed homozygosity for three autosomal chromosomes (6, 18, and 19) and a sex chromosome, suggesting that this choriocarcinoma developed from the complete mole of a single sperm origin. These results, however, did not entirely exclude the possibility that the choriocarcinoma is heterozygous for other chromosomes, although 46,XX heterozygous moles, if causal, are very rare (about 2%) in complete moles.

About 1900 DNA clones providing restriction fragment length polymorphism were already reported at the Tenth International Workshop on Human Gene Mapping in 1989.[22] Among them, DNA fragments in the HLA class II region are especially useful for individual identification because they offer multiple informative bands.[23] Indeed, we could distinguish the members of the patients' families by differences in restriction fragment length polymorphism patterns with only one probe for the HLA class II gene. A series of restriction fragment length polymorphisms originated from variable numbers of tandem repeat sequences has also been found on several chromosomes.[24] Thus further restriction fragment length polymorphism analyses with these informative probes that exhibit multiple alleles would facilitate the identification of the pregnancies responsible for choriocarcinomas.

In this study the pregnancies responsible for two choriocarcinomas preceded by multiple pregnancies were successfully determined by restriction fragment length polymorphism analysis. Application of restriction fragment length polymorphism analysis to a broader group of patients would certainly be of benefit to further define the natural history of gestational trophoblastic disease.

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Transvaginal fetal echocardiography in early pregnancy: Normative data

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Fifty-two transvaginal ultrasonographic examinations were performed between 10.0 and 14.9 weeks' gestation for the purpose of documenting normal fetal cardiac anatomy. All standard cardiac projections could be obtained by gestational week 12, and many were imaged by week 11. The four-chamber view was visualized in 90% of fetuses at 12 weeks' gestation and in 100% of fetuses examined at 13 weeks. The aortic root in short axis projection and the left ventricle in long axis view could be imaged in 70% and 40% of fetuses, respectively, by 12 weeks' gestation. Aortic and pulmonary valves were first visualized at 12 weeks, as were five-chamber, ductus arteriosus, and aortic arch views. The mitral and tricuspid valves were resolved in 60% of fetuses by 11 weeks' gestation. This study of normal cardiac anatomy suggests that there may be significant potential for the diagnosis of many fetal cardiac anomalies during the late first and early second trimesters of pregnancy. (AM J OBSTET GYNECOL 1991;165:688-91.)

Key words: Pregnancy, prenatal diagnosis, first trimester, transvaginal ultrasonography, echocardiography

The evaluation of both normal and abnormal fetal anatomy in early pregnancy with transvaginal ultrasonography is under active study. A number of investigators[1-3] have suggested that the prenatal diagnosis of malformations may move into the first trimester because of the ability to resolve small fetal structures with the transvaginal technique. However, before anomalous development can be assessed, the ability to adequately visualize normal anatomy must be examined. The systematic investigation of detailed cardiac anatomy in the first and early second trimesters with this approach has not been described. This study explores the use of transvaginal ultrasonography in visualizing normal early fetal heart anatomy.

Material and methods

Forty-two women at between 10 and 14.9 weeks' gestation were enrolled in this investigation. One patient was excluded because a missed abortion was documented at the time of ultrasonographic examination. Eleven patients were studied twice at two different gestational ages. A total of 52 transvaginal ultrasonographic examinations were available for analysis. All cardiac examinations were videotaped for purposes of documentation and later inspection.

Five gestational age ranges were defined for evaluation, in 1-week intervals from 10.0 to 14.9 weeks (10.0 to 10.9 weeks, 11.0 to 11.9 weeks, etc.). The gestational age at the time of examination was determined in each patient by either a crown-rump measurement or a biparietal diameter measurement with the use of established norms.[4, 5]

Fifteen echocardiographic views of the heart were selected for study because of their known general usefulness.[6, 7] Because at these early gestational ages the resolution of fine detail within the fetal heart might be insufficient to adequately visualize important aspects of

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Table I. Criteria required to accept adequate visualization of cardiac views

View	*Criteria*
Four-chamber	Two equally sized ventricles and atria; offset mitral and tricuspid valves; ventricular septum intact up to the cardiac crux
Five-chamber	Two equally sized ventricles and atria; discrete circular aortic cross section in center of heart
Aortic arch	Longitudinal arch view with at least one carotid, brachiocephalic, or subclavian branch clearly arising from the vessel
Short axis aorta	Discrete circular echo representing the aortic root visualized within the center of the heart
Short axis ventricles	Isolated right and left ventricular cavities in cross section with defined interventricular septum
Long axis left ventricle	Defined continuity from interventricular septum through the aortic root in longitudinal (parasagittal) section; mitral leaflet approximated to contralateral aortic wall
Inferior, superior vena cavae	Longitudinal views with vessel entrance into right atrium
Pulmonary trunk	Longitudinal view with definitive connection to right ventricle
Mitral, tricuspid valves	Leaflets as visualized on four-chamber view but interventricular septum not necessarily imaged
Aortic, pulmonary valves	Moving echogenic points near root of vessel in longitudinal or cross-sectional view
Ductus arteriosus	Continuity of pulmonary trunk into descending aorta; no emerging vessels projecting toward neck
Short axis base	Short axis view at level of aortic and pulmonic outflow tracts (pulmonary trunk draping over central aorta in cross section); tricuspid valve and right atrium in view

Table II. Percentage of cardiac structures visualized at each gestational age

View	*10-10.9 wk (n = 8)*	*11-11.9 wk (n = 10)*	*12-12.9 wk (n = 10)*	*13-13.9 wk (n = 13)*	*14-14.9 wk (n = 11)*
Four-chamber	0	30	90	100	100
Five-chamber	0	0	10	54	64
Aortic arch	0	0	20	23	73
Short axis aorta	0	10	70	62	82
Short axis ventricles	0	10	40	31	27
Long axis left ventricle	0	20	40	46	55
Inferior vena cava	0	10	10	54	91
Superior vena cava	0	10	10	23	36
Pulmonary trunk	0	0	40	38	82
Mitral valve	25	60	90	100	100
Tricuspid valve	25	60	90	100	100
Aortic valve	0	0	20	46	64
Pulmonary valve	0	0	20	15	36
Ductus arteriosus	0	0	50	23	73
Short axis base	0	10	30	46	73

anatomy, strict criteria were followed when documenting that a particular cardiac view was indeed imaged. These criteria are described in Table I.

Each ultrasonographic examination took no longer than 10 minutes to perform. An RT 3600 ultrasonography unit (General Electric, Rancho Cordova, Calif.) equipped with a 7.5 MHz transvaginal sector probe was used at double magnification. The study protocol was approved by the hospital's institutional review board for medical research. Written patient consent was obtained in all cases.

Results

Table II itemizes the cardiac views distinguished at each gestational age interval. A complete four-chamber view was consistently obtained by 12 weeks' gestation (in 90% of fetuses). This view was always obtainable by week 13. It was never adequately visualized at 10 weeks' gestation because an intact interventricular septum could not be completely resolved at this early age.

The five-chamber and aortic arch views were not seen in most fetuses until 14 weeks' gestation, although each was imaged in some fetuses by week 12. The resolution of the instrumentation limited consistent identification of the small carotid and subclavian vessels branching from the aorta up toward the neck before 12 weeks' gestation. Definitive identification of the ductus arteriosus in longitudinal view was similarly difficult until 14 weeks' gestation because the origin of tiny neck vessels could not always be established. In contrast, the aorta in short axis was well-defined by 12 weeks' gestation (in 70%), seen as a bright circular echo within the center of the heart. Structural detail was sufficient to relate this cross-sectional aortic view to the pulmonary trunk as a short axis base view in 46% of the fetuses by 13 weeks' gestation.

In 40% of cases a longitudinal (parasagittal) left ventricular view could be imaged by week 12. Earlier than this, the continuity between the aorta and the interventricular septum could not be reliably depicted in most fetuses.

The inferior and superior vena cavae were seen as early as 11 weeks' gestation. The aortic and pulmonary valves were first noted at 12 weeks' gestation, although even at 14 weeks they were not always discovered because of their small size. The complete short axis base view was first imaged at 11 weeks' gestation. The short axis ventricle view was also first visualized at 11 weeks but was inconsistently noted throughout the gestational ages studied.

No structural anomalies were noted in any of the fetuses studied. Because of a history of congenital heart disease or diabetes, eight patients had a formal level II fetal echocardiographic study at 18 to 22 weeks' gestation and in none of these were any cardiac malformations documented. The perinatal outcome of all patients was reviewed. Thirty-eight patients were delivered of infants with no clinical evidence of heart disease. One patient had a spontaneous abortion of unknown cause at 14 weeks' gestation. Another patient had a fetal loss at 22 weeks' gestation because of an incompetent cervix; the fetal heart on autopsy was normal. One patient had a pregnancy termination at 22 weeks' gestation because of bilateral renal agenesis, and an autopsy showed a normal fetal heart.

Comment

Echocardiographic investigation of the fetal heart has become a specialized examination of great importance inasmuch as congenital heart disease remains the most common serious anomaly at birth. The examination is usually performed at about 18 to 22 weeks' gestation. At this time the traditional transabdominal approach has sufficient resolution to delineate the small details of human heart anatomy. Earlier confirmation of normal cardiac anatomy is desirable to allay the anxiety of patients with a family history of congenital heart disease or to offer the option of early pregnancy termination or close follow-up should a cardiac anomaly be documented.

With the advent of high resolution transvaginal ultrasonography there is increasing interest in the analysis of normal and abnormal fetal anatomy during the first and early second trimesters of pregnancy.[1-3] Timor-Tritsch et al.[8] described a variety of fetal anatomic features detectable in 38 patients scanned with a 6.5 MHz transvaginal probe. They identified and described head, skeletal, and facial anatomy during the first trimester but structural cardiac details were not discussed in their survey. Kushnir et al.[9] studied first-trimester fetal cranial anatomy in 50 women by means of the transvaginal approach. They visualized many intracranial structures traditionally imaged only in the second trimester by the transabdominal route. Guzman[10] has reported the detection of gastroschisis at 12 weeks' gestation in an obese patient who was otherwise difficult to examine transabdominally. Cullen et al.[3] reported 33 fetuses between 9 and 13 weeks' gestation with structural anomalies, none of which were cardiac in nature. They suggested that with transvaginal ultrasonography, prenatal diagnosis of fetal anomalies would shift into the first trimester.

The only published report of diagnostic transvaginal fetal echocardiography to date is that of Gembruch et al.[11] They used a 5 MHz transvaginal probe to diagnose a complete atrioventricular canal defect and complete heart block at 11 weeks' gestation and subsequently performed an elective abortion on the basis of this information. They presented unpublished data suggesting that a four-chamber view was obtainable in 10 of 10 fetuses at 12 weeks' gestation and in 8 of 10 fetuses at 11 weeks' gestation. Our findings are generally supportive of their data, although with the use of strict criteria for the four-chamber view we could not image this projection in every fetus until 13 weeks' gestation. Echo dropout artifact obscuring the interventricular septum was invariably the reason for failure to visualize the complete four-chamber view at 10 to 12 weeks' gestation. Still, 90% of fetuses had a well-documented four-chamber view by 12 weeks. In contrast with this finding, Catanzarite and Quirk[12] could only establish an optimal four-chamber view on transabdominal examination in <20% of fetuses at 13 to 14 weeks' gestation. The importance of a normal four-chamber view has been emphasized by Copel et al.,[13] who showed that if this view on transabdominal ultrasonographic study was normal, >90% of congenital heart anomalies could be ruled out. Our data suggest that screening for congenital heart disease with the four-chamber view might be started as early as 12 to 13 weeks' gestation with the transvaginal approach.

It would appear that the earliest defined cardiac structures visible in the first trimester are the mitral and tricuspid valves. These are highly echogenic by week 10 to 11 and their range of motion is greater than any other intracardiac component. Fetal heart motion noted before 10 weeks' gestation most likely represents the movement of these two valves. Our data show that each atrioventricular valve can be individually resolved by 10 weeks' gestation in 25% of cases, and their normal offset position (tricuspid more apical than mitral) can be defined at this time. It was noted that these valves are usually observed before the interventricular septum can be identified.

Especially prominent by week 12 is the short-axis view of the aortic root, noted in 70% of fetuses at this age. The pulmonary trunk and the long parasagittal left ventricular view can also be seen in many cases by 12 weeks' gestation. These views in conjunction with the four-chamber projection, if normal in appearance, have the potential to rule out most structural defects by the third month of pregnancy.

This study illustrates the great quantity of fetal cardiac information available with the use of transvaginal ultrasonography in early pregnancy. However, some caveats concerning early cardiac diagnosis must be cited. Certain planes of view may be limited by the fixed linear axis of the ultrasonographic probe during the transvaginal examination, and fetal position often dictates the views that can be imaged. There is no information yet available concerning the sensitivity, specificity, and predictive value of first-trimester ultrasonographic cardiac diagnosis. Furthermore, considerable experience with second-trimester fetal echocardiography is necessary before attempting first-trimester diagnosis because magnification and resolution are limited in early pregnancy via the transvaginal route. Echo dropout artifact of small intracardiac structures (e.g., the interventricular septum in early four-chamber views) may lead to erroneous diagnosis of cardiac disease, so appropriate confirmation of a structural anomaly might necessitate serial ultrasonographic examinations. Some structural cardiac defects, such as aortic or pulmonic stenosis and corrected transposition of the great arteries, are exceedingly difficult or impossible to detect even with late second-trimester diagnosis.[14] These malformations will also not be amenable to first-trimester diagnosis. This study has documented that virtually all standard cardiac views can be visualized with the transvaginal approach by 13 weeks' gestation, and many can be obtained as early as 11 weeks. On the basis of these observations of normal cardiac anatomy it would appear that late first-trimester and early second-trimester diagnosis of congenital heart disease should be possible.

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Effects of angiotensin II on atrial natriuretic peptide in pregnancy

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To clarify the effects on angiotensin II and blood pressure on plasma atrial natriuretic peptide in pregnancy, angiotensin II was infused in 37 healthy, normotensive pregnant women in week 32 of gestation. Levels of atrial natriuretic peptide were determined with the women in a sitting position 3 days before the test and in the left lateral recumbent position immediately before and after the test. Levels of atrial natriuretic peptide were not influenced by parity or salt intake. They were found to be significantly higher in the left lateral recumbent position than in the sitting position. There was also a significant increase in atrial natriuretic peptide levels during angiotensin II infusion. A dose of angiotensin II <1.5 ng/kg/min did not affect atrial natriuretic peptide levels. Rise in atrial natriuretic peptide levels was minimally correlated with rise in mean arterial pressure, as caused by acute vasoconstriction from angiotensin II infusion. Therefore it is suggested that, in pregnancy, the response of atrial natriuretic peptide to angiotensin II is mediated not only by changes in blood pressure but also by angiotensinergic receptor activity in the atria. (AM J OBSTET GYNECOL 1991;165:692-5.)

Key words: Pregnancy, human; atrial natriuretic peptide; angiotensin II infusion

Atrial natriuretic peptide is a hormone with potent natriuretic and diuretic effects in man. The release of atrial natriuretic peptide by the heart is stimulated when pressure in the right or left atrium is raised.[1-3] Atrial natriuretic peptide is thought to respond primarily to increases in central blood volume,[4] acting in a homeostatic fashion to return central blood volume to normal and decrease blood pressure. Notwithstanding vast changes in the plasma volume and cardiac output in pregnancy, no (conclusive) evidence of a significant rise in atrial natriuretic peptide in normotensive pregnancies has been published. In preeclampsia, plasma volume and right atrial pressure are lower than in normal pregnancies.[5,6] The paradox, however, is that in preeclampsia the atrial natriuretic peptide concentration (notwithstanding its natriuretic and diuretic power) is higher than in normal pregnancy.[7]

In support of the hypothesis that high atrial natriuretic peptide levels are secondary to elevated angiotensin II levels, pressor infusions of angiotensin II have been found to raise plasma atrial natriuretic peptide levels both in nonpregnant subjects[8,9] and in pregnant ones.[10] Other investigators did not confirm this influence of angiotensin II infusions on atrial natriuretic peptide levels in plasma.[11] It seems that central blood volume and plasma angiotensin II can independently affect atrial natriuretic peptide levels.[12]

In pregnancy the angiotensin II pressure test is a good predictor of a forthcoming pregnancy-induced hypertension.[13-16] Regarding atrial natriuretic peptide, the response to angiotensin II may be mediated by changes in blood pressure levels, increased angiotensin receptor activity in the atria, or both.[9] To elucidate this point during pregnancy, the effects of subpressor and pressor infusions of angiotensin II on plasma atrial natriuretic peptide were investigated.

Patients and methods

Thirty-seven women, all participating in the "Salt restriction in pregnancy" project, gave informed consent to the study protocol, which was approved by the hospital's ethical committee. They were women with an ultrasonography-dated, singleton pregnancy, a mean age of 29.75 years (22.95 to 36.64) and a mean weight of 74.0 kg (52.3-111.4).

None of the nulliparous women ($n = 22$) had a history of renal disease or a personal history of hypertension, whereas all of the parous women ($n = 15$) had a personal history of pregnancy-induced hypertension, preeclampsia, stillbirth, or abruptio placentae. In the first trimester of pregnancy all patients were divided at random into one group with a free-choice diet and another one that followed a strongly sodium-restricted

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diet. The diet was considered strongly sodium restricted if sodium excretion in several 24-hour urine samples throughout pregnancy was consistently <30 mmol/24 hr. The diet was considered not sodium restricted if sodium excretion in 24-hour urine samples exceeded 60 mmol/24 hr. If sodium excretion in the 24-hour urine samples was >30 but <60 mmol/24 hr, the diet was considered intermediately sodium restricted (Table I).

In week 32 of gestation, up to 3 days before the angiotensin II infusion, venous blood samples were collected for atrial natriuretic peptide determination from 26 of the total population of 37 normotensive (diastolic blood pressure <85 mm Hg) pregnant women while they were in a sitting position. The fully standardized angiotensin II infusion test of Gant et al.[13] was modified as follows: The patient was placed in the left lateral recumbent position with an intravenous line in the right arm. A 5% dextrose solution was administered by drip. The intravenous system could be switched to another 5% dextrose solution containing angiotensin II (Hypertensin, Ciba-Geigy Ltd., Basel) in a concentration of 1 μg/ml. The rate of infusion of the angiotensin II solution was regulated by an infusion pump.

When a steady state in blood pressure levels (with an automatic recorder [Dinamap vital signs monitor model 1846, Critikon Inc., Tampa] on the left arm) was reached, venous blood samples were collected for atrial natriuretic peptide determination just before and after the angiotensin II infusion.

The angiotensin II solution was started at a rate of 3 ng/kg/min after a constant diastolic blood pressure had been measured during a period of at least 15 minutes; the rate of infusion was raised every 4 minutes by 1 ng/kg/min. The test was considered positive if the effective pressure dose (the dose of angiotensin II required to elevate baseline diastolic blood pressure 20 mm Hg) was <12 ng/kg/min. Angiotensin II administration was stopped when the diastolic blood pressure had risen 20 mm Hg or the dose of angiotensin II had reached 12 ng/kg/min, although Gant et al. continued infusion at higher doses until the effective pressor dose was reached.

Four of the 37 normotensive pregnant women received a sub-subpressor infusion of angiotensin II (1.2 ng/kg/min). A subpressor infusion of angiotensin II (12 ng/kg/min) was given to 28 women, increasing diastolic blood pressure from −5 to 19 mm Hg. Pressor infusions of angiotensin II (<12 ng/kg/min) were given to five women, increasing diastolic blood pressure 20 mm Hg. All tests were performed in the morning to avoid the influence of diurnal variations of plasma levels of endogenous angiotensin II and atrial natriuretic peptide.[17] For the same reason the blood sample up to 3 days before the test was collected in the morning. Plasma immunoreactive atrial natriuretic peptide was measured by radioimmunoassy as previously described.[18] Blood was collected in chilled polystyrene tubes containing potassium and ethylenediaminetetraacetic acid (1.5 mg/ml blood) and aprotinin (Trasylol, 200 kIU/ml blood). Blood was kept on ice and centrifuged (4° C) as soon as possible. Plasma was divided in portions and stored at −80° C. Before the assay, plasma was extracted on SepPak C18 reverse phase columns (Waters Associates, Milford, Mass). The antibody in the radioimmunoassay was elicited against α-human atrial natriuretic peptide (1-28) in sheep; the tracer hormone was iodine 125–monoiodotyrosyl–human atrial natriuretic peptide (1-28), purified by means of aqueous phase liquid chromatography. The separation of bound and free hormone was performed by horse second antibody against sheep, coupled to solid-phase radioimmunoassay. The recovery of human atrial natriuretic peptide (1-28) that was added to plasma was 90% ± 5%. The sensitivity was 0.8 pg per tube (8 pg/ml plasma). The interassay and intraassay precisions were 12% and 9% respectively. Statistical analyses were performed with the Wilcoxon signed rank test and the Mann-Whitney test. Levels of significance were $p < 0.05$. Results were expressed as median and range. Presence or absence of increase was determined by the sign test, and correlation coefficients were calculated.

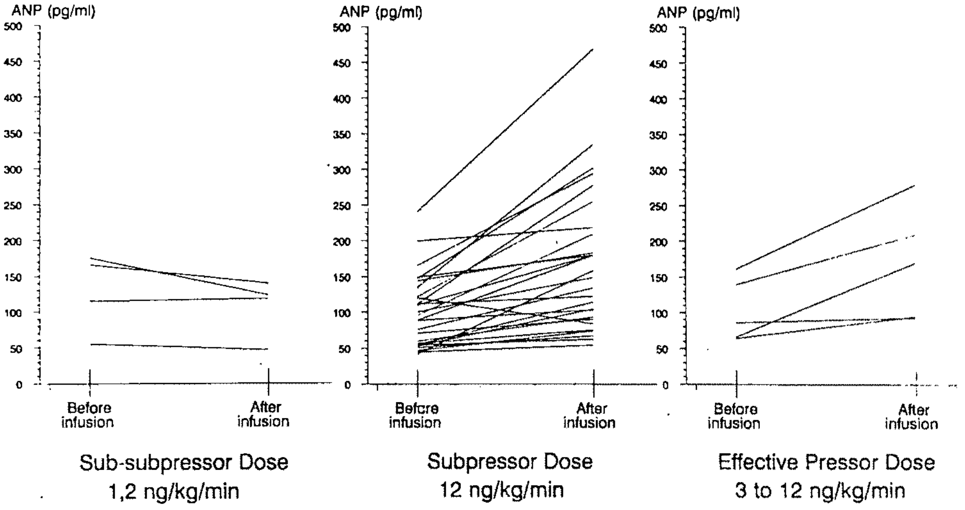
Table I. Number of nulliparous and parous patients according to dietary sodium restriction.

	Nulliparous	*Parous*	*Total*
Sodium restricted (<30 mmol/24 hr)	1	8	9
Intermediate sodium restricted (30-60 mmol/24 hr)	0	1	1
Not sodium restricted (>60 mmol/24 hr)	21	6	27
TOTAL	22	15	37

Results

All normotensive women had lower atrial natriuretic peptide levels while they were in a sitting position than just before the start of the angiotensin II infusion while they were in left recumbent position (sitting, 50.5 pg/ml, range 27 to 86; before test, 100 pg/ml, range 42 to 240; $p < 0.0001$). There were no differences between nulliparous and parous women or between sodium restricted and free-choice diets.

Fig. 1 shows that 45 minutes of sub-subpressor angiotensin II infusion (1.2 ng/kg/min) in the four normotensive pregnant women did not increase atrial natriuretic peptide levels (before test, 139.5 pg/ml, range



Sub-subpressor Dose
1,2 ng/kg/min

Subpressor Dose
12 ng/kg/min

Effective Pressor Dose
3 to 12 ng/kg/min

Fig. 1. Atrial natriuretic peptide before and after infusion of sub-subpressor dose (1.2 ng/kg/min), ubpressor dose (12 ng/kg/min), or effective pressor dose (3 to 12 ng/kg/min and rise in diastolic blood pressure of ≥20 mm Hg) of angiotensin II.

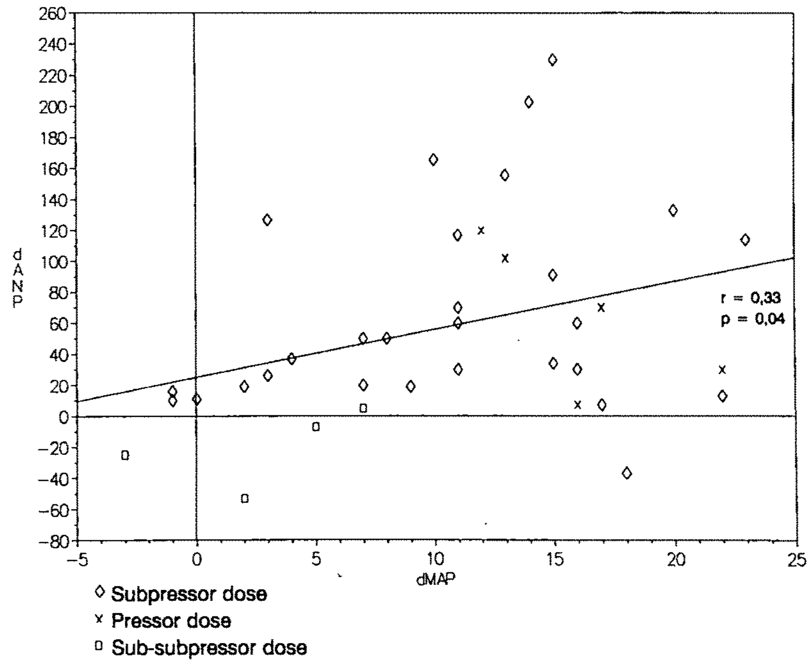


Fig. 2. Relationship between change in mean arterial pressure (*dMAP*, millimeters of mercury) and change in plasma atrial natriuretic peptide (*dANP*, picograms per milliliter) during angiotensin II infusion for sub-subpressor dose (□), subpressor dose (◇), and effective pressor dose (×).

54 to 175; after test, 120.5 pg/ml, range 47 to 140). However, subpressor infusions of angiotensin II at a maximum level of 12 ng/kg/min in 28 women significantly increased atrial natriuretic peptide levels (before test, 98 pg/ml, range 42 to 240; after test, 154.5 pg/ml, range 55 to 470; $p < 0.0001$). After angiotensin II pressor infusion (4 to 12 ng/kg/ml) the atrial natriuretic peptide levels rose significantly, from 85 pg/ml (range 64 to 160) to 170 pg/ml (range 92 to 280) ($p < 0.05$). In spite of a tendency toward a correlation between an increase in mean arterial pressure and an increase in atrial natriuretic peptide levels during angiotensin II infusions, this tendency did not reach statistical significance ($r = 0.33$; $p = 0.04$) (Fig. 2).

Comment

The role of atrial natriuretic peptide in blood pressure regulation and the role of blood pressure in atrial natriuretic peptide regulation in pregnancy is not yet fully understood. The release of atrial natriuretic peptide may be controlled by a number of mechanisms, such as enhanced receptor-mediated responses that are caused by known vasoconstrictor agents such as angiotensin II and a rising pressure in the atria. A trivial activity like a change from sitting to recumbent position causes a higher atrial pressure and a higher atrial natriuretic peptide level.[19, 20] In accordance with this, the atrial natriuretic peptide levels increased significantly when the normotensive pregnant women were lying in a left lateral position.

It should not be surprising that, during pregnancy, the atrial natriuretic peptide levels are significantly elevated by pressor angiotensin II infusions. The test of Gant et al. is based on an increase in diastolic blood pressure by 20 mm Hg. So the resulting acute vasoconstriction of the arteriolae will affect, in one way or the other, the atrial stretching and thus the atrial natriuretic peptide levels. In the nonpregnant situation a close relationship between a change in mean arterial pressure and a change in plasma atrial natriuretic peptide during angiotensin II infusions has been established.[9]

However, in this study elevated atrial natriuretic peptide levels were found not only during pressor angiotensin II infusions but also during subpressor angiotensin II infusions. Moreover, the close correlation between a change in mean arterial pressure and a change in plasma atrial natriuretic peptide during angiotensin II infusions seems to have vanished in pregnancy.

The study design itself could elevate the atrial natriuretic peptide levels, but a lack of change in atrial natriuretic peptide during the sub-subpressor angiotensin II infusions in this study is contradictory to that idea. The results of this study lead to the hypothesis that during pregnancy the response of atrial natriuretic peptide to angiotensin II may be mediated by changes in blood pressure, as well as by angiotensinergic receptor activity in the atria.

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Expression of cytokine receptors and markers of differentiation in human papillomavirus–infected cervical tissues

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Human papillomavirus infection of the uterine cervix is associated with a spectrum of benign, premalignant, and malignant epithelial lesions, a process that appears to require the coordinated effects of secondary cellular and environmental events. We have used flow cytometry and immunohistochemistry to examine the expression of the cellular markers for proliferation (interleukin-1, epidermal growth factor receptor, and transferrin receptor) and the markers of cellular differentiation (filaggrin and low-molecular-weight cytokeratin) in normal and human papillomavirus–infected human cervical tissues representing the natural range of human papillomavirus–induced disease. The results were correlated with the histologic grade of disease, human papillomavirus type, cellular deoxyribonucleic acid content, and cell cycle status. Interleukin-1 and transferrin receptor were slightly increased in high-grade dysplasias and in squamous cell carcinomas. Filaggrin expression was found to be inversely related and cytokeratin and epidermal growth factor receptor expression directly related to the degree of neoplasia. These findings indicate that cytokeratin and epidermal growth factor receptor are useful markers of cell proliferation in human papillomavirus–infected tissues and that their expression may directly increase as a result of infection. (AM J OBSTET GYNECOL 1991;165:696-705.)

Key words: Human papillomavirus deoxyribonucleic acid, growth factors, cellular differentiation, flow cytometry

Human papillomavirus (HPV) infection of the uterine cervix is associated with the development of a spectrum of benign and malignant epithelial lesions, on the basis of the fact that viral deoxyribonucleic acid (DNA) is detected in these lesions in the majority of cases.[1] Although the epidemiologic data support a role for HPV in the development of cervical neoplasia, it is generally regarded that HPV is not a sole causative agent but rather may be an initiating event in what is considered to be a multistep process of neoplastic transformation.[2]

A continuum of morphologic changes occurs in cervical tissue infected with HPV that is marked by the gradual increase in cellular dysplasia, increased mitotic activity, microinvasiveness, and loss of specific viral cytopathic features such as koilocytosis and viral capsid antigens.[3] There are also a host of biochemical alterations at the nuclear, cytoplasmic, and plasma membrane level that also take place. The expression of cytoskeletal proteins such as cytokeratin and of differentiation-dependent cytoplasmic proteins such as filaggrin and involucrin are markedly altered in cells containing HPV DNA.[4,5] These markers can be used to characterize the neoplastic state. However, quantification is not possible on the basis of current methods. As we show below, flow cytometric analysis permits accurate and sensitive quantification of these parameters.

Similar methods can be used to determine the proliferative activity of cells containing HPV DNA. There are more than 50 subtypes of HPV, yet only a few such as HPV types 16, 18, and 31 DNAs are consistently found in malignant tumors, whereas several nononcogenic viral types such as HPV types 6 and 11 were usually, but not exclusively, associated with benign lesions.[6] The nature of the differences between these viruses in their ability to impart cellular transformation is unknown but is purported to be due to variations in their genomic organization, levels of viral gene transcription, and functional activity of the virus-derived oncoproteins.[7] The physical state of the viral DNA within cells, whether episomal or integrated into the host chromosomes, has been shown to be an important predictor of malignant progression.[8] HPV by itself, however, does not appear to be able to induce cellular transformation. In vitro studies examining the capacity of HPV DNA to transform primary cells in culture illustrate that HPV must act in concert with secondary

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cellular or host factors, such as the activated oncogenes Ha-*ras* to achieve the fully transformed phenotype.[9]

A number of factors intrinsic to the host may contribute to the process of cervical tumorigenesis, including extracellular factors such as steroid hormones or the integrity of the immune system.[10, 11] In addition, several studies have examined the role of cellular cytokines in cellular transformation in non-HPV-infected cells, demonstrating in particular how certain growth factors and their receptors may function as tumor promotors and enhance the process of cell transformation. The expression of growth factor receptors has been shown to be clinically important in the assessment of tumors in patients and appears in some cases to portend prognostic significance.[12] Recently, there has been evidence to suggest that an increased expression of transferrin or epidermal growth factor receptors is linked to progression of HPV disease.[13] In vitro studies corroborate these results, showing that immortalized cell lines containing HPV type 16 DNA have an altered responsiveness to growth factors such as estrogen, progesterone, and epidermal growth factor.[14] This study was designed to examine the role of several cytokines such as cytoplasmic interleukin-1 (IL-1) and cytokine receptors for epidermal growth factor and transferrin in a series of HPV-associated cervical lesions, by determining whether the expression of these markers correlates with the presence of HPV DNA, the level of cellular differentiation, DNA ploidy, cell cycle analysis, and histopathologic features.

Material and methods

Tissue collection. Samples of cervical cells were obtained from a series of women visiting a local medical center. The cervix of each patient was swabbed with 3% acetic acid and visualized with a colposcope. Sampling was performed by a gynecologic oncologist (L.F.C.) with a Cytobrush (International Cytobrush, Inc., Hollywood, Fla.) directed to areas suspicious for HPV infection, followed by a biopsy with 3 mm biopsy forceps. The exfoliated cell samples were transported in cold RPMI 1640 media (Gibco, BRL, Gaithersburg, Md.) containing penicillin (200 U/ml), streptomycin (200 U/ml), and amphotericin B (Fungizone) (5 μg/ml) and refrigerated. The portion of the samples used for flow cytometry was processed within 3 hours of collection, first by centrifugation to pellet the cells, then by repeated washings with 10 ml of cold phosphate-buffered saline solution. The samples were resuspended in 5 ml phosphate-buffered saline solution and treated with type II collagenase and Dispase (Boehringer-Mannheim, Indianapolis) at a final concentration of 10 mg/ml and incubated at 37° C for 30 minutes. The samples were further disaggregated with gentle pipetting, resulting in a uniform cell suspension. Cell viability was routinely 85% to 90%. The biopsy samples were frozen in liquid nitrogen and later routinely processed after formalin fixation and paraffin embedding.

DNA analysis by Southern blot. A portion of the exfoliated cervical cells was extracted for total cellular DNA by pronase treatment, phenol extraction, and ethanol precipitation, as described previously.[6] Total cellular DNA from each sample (5 μg) was digested with the restriction enzyme *Bam*HI, and subjected to one-dimensional gel electrophoresis as previously described.[6] Electrophoresed cellular DNA was denatured in alkali, neutralized, and transferred to a nylon membrane (Zeta-bind, BRL, Boston). Filters were prehybridized and hybridized as previously described, probed with 5 ng/ml random-primed HPV plasmid DNA (specific activity, 4 to 6 × 10^8 cpm/μg), and washed under very-high-stringency conditions (−4° C), which can differentiate HPV type 6 from HPV type 11.[6, 15] Damp filters were autoradiographed with Kodak XAR-5 x-ray film (Eastman Kodak, Rochester, N.Y.) and intensifying screens at −70° C. HPV types 6, 11, 16, and 18 plasmid DNAs were a generous gift from Drs. L. Gissmann and H. zur Hausen, HPV type 31 was from Dr. A. Lorenz, and HPV type 33 from Dr. G. Orth.

Flow cytometric analysis. Disaggregation and preparation of a uniform suspension of ectocervical cells were performed as described previously.[16] Samples were stained for surface and cytoplasmic markers and for total cellular DNA, by means of a modification of a procedure described previously.[17] Briefly, samples were washed twice with cold phosphate-buffered saline solution and resuspended in cold phosphate-buffered saline solution. Totals of 1 to 2 million cells were aliquoted for each staining reaction, which was performed in sterile tubes in a final volume of 120 μl. Unfixed cells were stained with the monoclonal antibodies for transferrin receptor (Becton-Dickinson, Mountain View, Calif.), or epidermal growth factor receptor (Bioscience Products Inc., Indianapolis). Aliquots of cells fixed in cold 50% ethanol were stained with the markers filaggrin (Biomedical Technologies Inc., Stoughton, Mass.), cytokeratin (clone CAM 5.2) (Becton-Dickinson), and IL-1 (Olympus Immunochemicals, Lake Success, N.Y.). All primary antibodies were monoclonal murine antihuman reagents commercially labeled with either fluorescein isothiocyanate or phycoerythrin. Antibodies were incubated with cells at room temperature for 40 minutes, followed by two washes with phosphate-buffered saline solution. Samples and normal peripheral blood lymphocytes were stained for each of the respective markers and then compared with samples with nonspecific staining by fluorochrome-labeled IgG1 murine sera (Becton-Dickinson). In an effort to minimize the detection of intermixed cells and cellular fragments, the measurement of mean fluorescence of these markers was standardized by electronically selecting (gating) only those cells with mean cell diameter > 20 μm. Propidium iodide staining for total cellular DNA content was performed on ethanol-fixed cells as previously described.[16, 17] Analyses were performed on a Becton-Dickinson FACS 440 flow cytometer with a 10 mW helium-neon laser.

Histopathology and immunohistochemistry. Standard sections were stained with hematoxylin and eosin; the histologic diagnoses were reviewed by the authors (R.C.M. and M.J.S.) and corroborated by an independent pathologist. The diagnoses were classified according to standard nomenclature for grading cervical dysplasia, as initially proposed by Richart.[18] Each sample was subsequently stained with the markers used for flow cytometry according to methods of immunohistochemistry as previously described.[19] Primary antibodies were applied dropwise to deparaffinized tissue sections to a final dilution of 1000 : 1 to 2000 : 1 and incubated overnight at 4° C. The secondary biotin-conjugated horse antimurine antibody was incubated for 1 hour at room temperature. Appropriate positive control tissues were prepared with each staining procedure.

Results

Patient samples and detection of HPV DNA by Southern blot. Thirty-four women who were seen at the Gynecology Clinic of Hennepin County Medical Center in Minneapolis were included in this study. Biopsy and cervical scrape samples of the uterine cervix were analyzed for the expression of cellular markers of proliferation and differentiation by flow cytometry. These data were correlated to the histologic diagnosis and results of Southern blot analysis to detect viral DNA. Efforts were made to select patients whose diagnoses covered a broad range of clinical manifestations of HPV-associated cervical diseases, ranging from normal to severe dysplasia and squamous carcinoma (Table I). In this table and in subsequent figures, samples were grouped according to their histologic criteria and, while shown together, were not intended to represent a biologic continuum.

HPV infection of the cervix was determined by detection of HPV type-specific DNA by Southern analysis. Fig. 1 illustrates the serial hybridizations of filters with radiolabeled HVP DNA. A positive sample was identified as an 8 kb fragment, which is the unit length of the HPV genomes. Although many more types of HPV are known to exist in the cervix, the ones examined represent the types most frequently detected in anogenital lesions. In several samples (e.g., cases 11, 19, 24, 26, 29, and 32) bands other than 8 kb were observed, indicating integration events or deletion mutants of episomal DNA.

The correlation of histologic appearance of cervical samples with the detection of HPV DNA is listed in Table I. Overall, 24 of 34 patient samples were HPV DNA positive. No HPV DNA was detected in the eight biopsy samples with normal or nonspecific histologic types. In contrast, five of eight samples showing features of condyloma acuminatum or low-grade dysplasia were positive for HPV type 6 or 11, one case for HPV type 16, and two cases were HPV DNA negative. HPV DNA was detected in all 16 cases of moderate or high-grade dysplasia. HPV type 16 DNA was detected in 10 patient samples; one case of grade 1 cervical intraepithelial neoplasia, seven cases of grades 2 and 3 cervical intraepithelial neoplasia, and two invasive squamous cell carcinomas. HPV type 18 DNA was detected in six cases of grade 2 and 3 lesions. HPV type 31 DNA is infrequently detected in cervical dysplasias but was seen in three grade 3 cervical intraepithelial neoplasia lesions. No cases demonstrated hybridization with multiple HPV types.

DNA ploidy analysis of cervical cells stained with propidium iodide. Neoplastic transformation is associated with aneuploid populations of tumor cells in most de novo malignancies, a feature that can be detected and characterized by flow cytometry on cell samples stained for total cellular DNA.[20] In addition, DNA aneuploidy has been shown to correlate with higher-grade histologic diagnosis, the expression of cell proliferation antigens, and the loss of expression of papillomavirus capsid antigen; therefore it was examined in this study.[21]

DNA ploidy of the patient samples and normal peripheral blood lymphocytes was analyzed by flow cytometry after samples of fixed cells were stained with propidium iodide; the results are provided in Table II. The coefficient of variation, a measure of precision, was low for all samples and ranged between 3.2% and 7.0%. Five of the 34 cervical samples analyzed contained hyperdiploid G_1/G_0 populations of cells, which were distinct from the larger diploid population. One case of grade 1 cervical intraepithelial neoplasia, two of grade 3 cervical intraepithelial neoplasia, and two of squamous cell carcinoma were found to be aneuploid, and each was positive for HPV DNA (Table II). Similarly, the DNA index, calculated as the ratio of the size and fluroescence of the hyperdiploid peak to the diploid peak of the reference cells (peripheral blood lymphocytes), ranged between 1.6 and 1.88 for the aneuploid samples.

The proliferative index is the percentage of cells in the $S + G_2/M$ phase and was calculated from samples stained for total cellular DNA. Samples of normal cer-

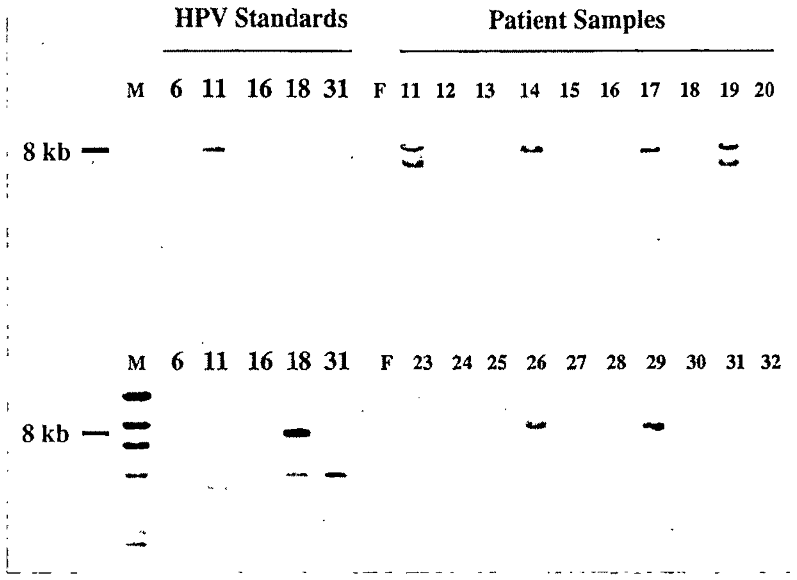
HPV Standards
Patient Samples
M 6 11 16 18 31 F 11 12 13 14 15 16 17 18 19 20
8 kb
M 6 11 16 18 31 F 23 24 25 26 27 28 29 30 31 32
8 kb

Fig. 1. Southern blot analysis of DNA extracted from patients' exfoliated cervical cell samples. Results of hybridization of radiolabeled purified HPV type 11 *(top)* or 18 *(bottom)* plasmid probes are shown. Plasmid portion of HPV type 18 probe detects vector bands for other corresponding plasmid controls. Plasmids containing HPV viral DNAs (10 pg) and *Bam*HI-digested neonatal foreskin DNA *(F)* were used as positive and negative controls, respectively. λ Phage DNA digested with *Hin*dIII *(M)* served as molecular weight markers.

Table I. Typing of HPV DNA from cervical scrape samples

	HPV type					
Biopsy diagnosis	*6/1*	*16*	*18*	*31*	*HPV DNA negative*	*Total*
Normal	0	0	0	0	8	8
Condyloma acuminatum	2	0	0	0	0	2
Grade 1	3	1	0	0	2	6
Grade 2	0	4	3	1	0	8
Grade 3	0	3	3	2	0	8
Squamous cell carcinoma	0	2	0	0	0	2
TOTAL	5	10	6	3	10	34

Genomic DNA (5 μg) from samples of cervical cells was extracted and digested with the restriction endonuclease *Bam*HI and then electrophoresed. The DNA was transferred to nylon membranes by the Southern blot method. Hybridization was performed with cloned HPV types 6, 11, 16, 18, and 31 under very-high-stringency conditions

vical epithelium had a mean proliferative index of 16%, which compares with the proliferative index of peripheral blood lymphocyte cells (15.5%) and increased to 26% in the grade 3 cervical intraepithelial neoplasia group. Samples in the grade 1 cervical intraepithelial neoplasia group had a slightly lower proliferative index (15%), which is not statistically significant from that of the normal samples ($p > 0.1$). There was also a positive correlation between the presence of HPV DNA as determined by Southern blot and an increased proliferative index ($p < 0.05$); this relationship was also found to hold for the group of samples containing the oncogenic HPV types 16, 18, and 31.

Markers of differentiation with histologic grade of dysplasia and HPV types. Infection of cervical tissues with HPV has been shown to alter the pattern of differentiation of squamous epithelium. Concomitant with this is altered expression of differentiation-dependent proteins, such as the cytokeratin family of intermediate filaments and filaggrin, a precursor to keratohyaline granules. A series of monoclonal antibodies have been developed for the identification of cytokeratins of varying molecular weights. Low-molecular-weight species of cytokeratin have been found in undifferentiated and malignant epithelium, in particular, squamous cell carcinoma.[4] In this study we have used the monoclonal antibody CAM 5.2 to detect the low-molecular-weight cytokeratin protein, to identify undifferentiated or

Table II. Correlation of histologic diagnosis with DNA ploidy, DNA index, and proliferative index of total cellular DNA stained by propidium iodide and analyzed by flow cytometry

Biopsy diagnosis	*Ploidy*	*No. of cases per total No.*	*DNA index**	*Proliferative index (% cells in S + G_2/M)*	
				Proliferative index	*Coefficient of variation*
Normal	Diploid	8/8	1.0	16	3.8
Condyloma acuminatum	Diploid	2/2	1.0	18	4.2
Cervical intraepithelial neoplasia					
Grade 1	Aneuploid	1/6	1.64	15	7.0
Grade 2	Diploid	8/8	1.0	21	4.5
Grade 3	Aneuploid	2/8	1.88	26	6.9
Squamous cell carcinoma	Aneuploid	2/2	1.60	25	3.7
HeLa	Aneuploid			31	3.2
CaSki	Aneuploid			35	3.1

*DNA index was calculated from data points corresponding only to aneuploid population of cells.

neoplastic exocervical cells. The cytoplasmic protein filaggrin is synthesized by normal squamous cells and normally sequestered in the superficial layers of squamous epithelium; in this study it was used as a marker of normal keratinocytic differentiation.[5]

Ethanol-fixed cells from cervical brushings of patients were stained for these markers of differentiation and measured by flow cytometry and correlated with their histologic grade and HPV type. Fig. 2 illustrates the relationship between the average expression of these two markers for the aggregate of patient samples showing various histologic groups of HPV-associated cervical lesions that we have studied. Cytokeratin was differentially expressed in normal and neoplastic tissue. Normal and grade 1 and 2 cervical intraepithelial neoplasia samples had measurable expressions of cytokeratin that were not significantly different from each other ($p > 0.1$). Both cases of condyloma acuminatum demonstrated slightly less bright staining for cytokeratin. By contrast, grade 3 samples were on average nearly twofold brighter than the lower-grade sample groups, which was not statistically significant ($p > 0.1$), but consisted of a single population of large cells (mean cell diameter, 20 μm). Both cases of squamous cell carcinoma strongly expressed cytokeratin that was twofold greater than that of the grade 3 cervical intraepithelial neoplasia samples ($p < 0.01$). The expression of cytokeratin was also analyzed in a series of cervical carcinoma cell lines that contain HPV DNA. Each of the cell lines HeLa (HPV 18) and CaSki (HPV 16) displayed strong expression. Although these cell preparations were not identical to those of the patient samples, the results were comparable to those in the cases of squamous carcinoma. No measurable staining of peripheral blood lymphocytes by cytokeratin was observed. Thus, as indicated below, these cells lines do in fact reflect many of natural physical traits of tumor cells in vivo and can be used for further similar studies.

The relationship of filaggrin expression to histologic type was found to be inversely related to the level of cellular differentiation. Samples of cervical tissues with normal histologic features demonstrated the highest level of filaggrin staining, followed by a marked decrease in the mean fluorescence of the cases of condyloma acuminatum. Examples of grade 1 cervical intraepithelial neoplasia cases had a mean fluorescence for filaggrin only slightly less than that for condyloma acuminatum, whereas samples of grades 2 and 3 cervical intraepithelial neoplasia showed a further decrease in filaggrin expression. Expression of filaggrin was very low but greater than background in the cases of squamous cell carcinoma and in the cervical carcinoma cell lines. Immunohistochemical staining of formalin-fixed tissue sections could qualitatively distinguish between normal and grades 1 and 2 cervical intraepithelial neoplasia lesions and between grade 3 cervical intraepithelial neoplasia and squamous cell carcinoma but could not quantitatively separate further between histologic groups (data not shown).

Expression of the proliferative cytokines epidermal growth factor and transferrin receptors and cytoplasmic IL-1. The expression of the proliferative markers epidermal growth factor receptor, transferrin factor receptor, and IL-1 and measured by flow cytometry is shown in Fig. 3. Transferrin receptor and cytoplasmic IL-1 had measurable fluorescence in all cases from all histologic groups, which increased only slightly in cases of squamous cell carcimona. The expression of transferrin receptor in cases of grade 3 cervical intraepithelial neoplasia and squamous cell carcinoma were at least twice as great as for the other histologic groups. IL-1 and transferrin receptor in Hela and CaSki cells were slightly less than those of the squamous cell carcinoma but were also greater than those of the low-grade and moderate-grade dysplasias.

The expression of epidermal growth factor receptor

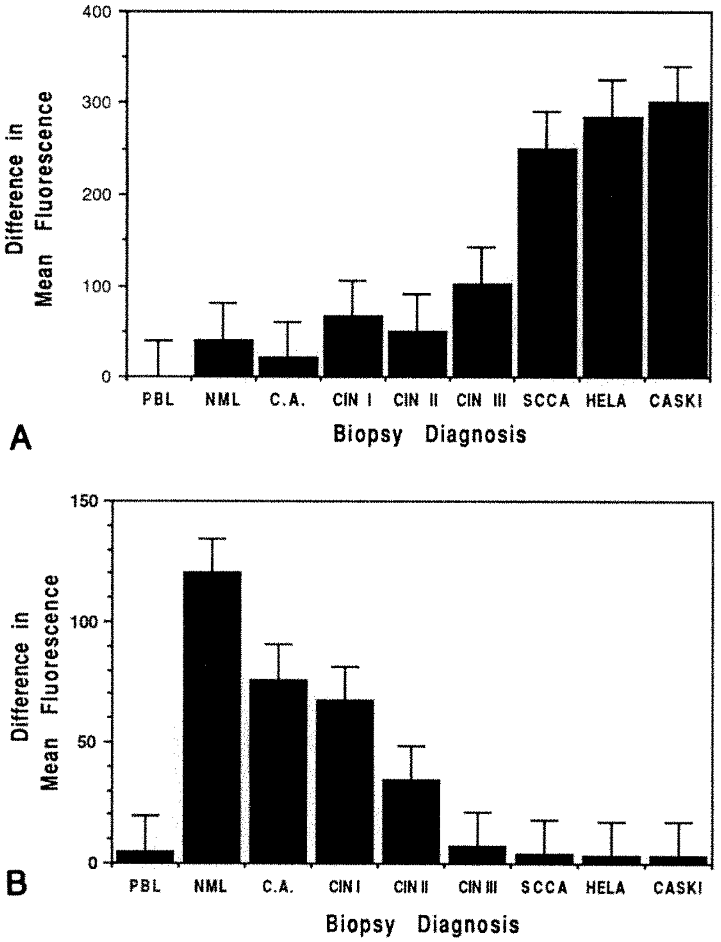

Fig. 2. Expression of structural cytoplasmic proteins cytokeratin (CAM 5.2) and filaggrin in exfoliated cervical tissue. Samples of cervical cells were stained with either antifilaggrin or anticytokeratin antibodies and analyzed by flow cytometry. Data presented are calculated from difference in mean fluorescence of sample from that of negative control: Sample − Control/Control. *Error bars* represent ± SEM. **A,** Cytokeratin expression. **B,** Filaggrin expression. *PBL,* peripheral blood lymphocyte; *NML,* normal; *CIN,* cervical intraepithelial neoplasia; *SCCA,* squamous cell carcinoma.

increased significantly in tissues showing higher grades of dysplasia and squamous cell carcinoma and in HPV-infected cell lines. The fluoresence for low-grade lesions (condyloma acuminatum and grade 1 cervical intraepithelial neoplasia) was twofold greater than that of normal samples, and the difference between these groups was not significant ($p > 0.1$). In the grade 3 cervical intraepithelial neoplasia and squamous cell carcinoma groups the relative expression levels of epidermal growth factor receptor were six to seven times those of normal samples ($p < 0.05$). These data demonstrate that the expression of epidermal growth factor receptor correlates with the severity of the histologic grade. Similarly, the expression of epidermal growth factor receptor in Hela and CaSki cells was comparable to that of the samples of squamous cell carcinoma.

In observations that were similar to those with cytokeratin and filaggrin, immunohistochemistry for epidermal growth factor receptor could only qualitatively distinguish low-grade from high-grade neoplasias; this is shown in Fig. 4, *A* and *B*. Immunohistochemistry was generally found not to be sensitive enough to show the differential staining intensity that was observed with flow cytometric analysis; these results are similar to those for the markers cytokeratin and filaggrin. Instead, tissue sections stained for epidermal growth factor receptor had no identifiable staining for either the normal or the condyloma acuminatum histologic group. An example of a grade 1 cervical intraepithelial neoplasia lesion is shown in Fig. 4, *A*, which demonstrates membranous localization of epidermal growth factor receptor in the proliferting basal and parabasal cells. Fig. 4, *B*, by contrast, reveals intense membranous outlining by the epidermal growth factor receptor protein, which was present in cells throughout the full thickness of the epithelium in grade 3 cervical intra-

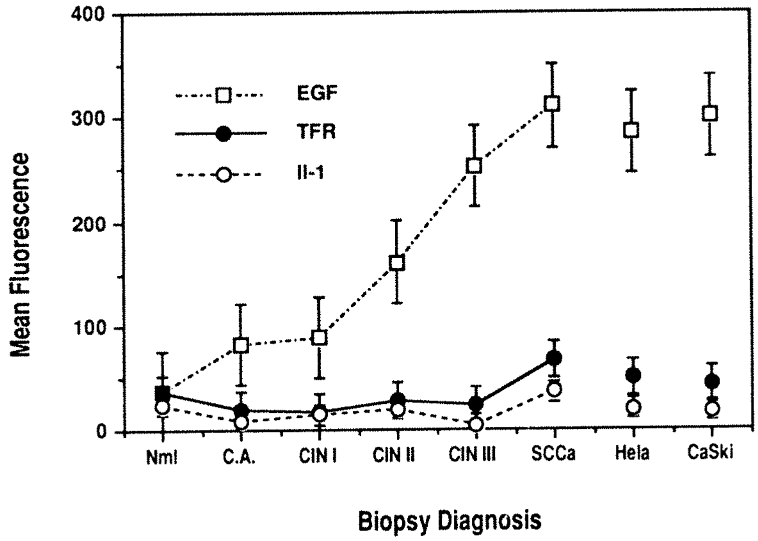

Fig. 3. Expression of cellular markers of proliferative epidermal growth factor receptor, transferrin receptor, and cytoplasmic IL-1. Shown on left are results from normal and neoplastic cervical cell samples *(points connected by lines)* and on right, from two cervical carcinoma–derived cell lines containing HPV DNA. Samples of cervical cells were stained with anti–epidermal growth factor receptor, anti–transferrin receptor, and anti–IL-1 and analyzed by flow cytometry. Data presented are calculated from difference in mean fluorescence of sample from that of negative control: (Sample − Control/Control). *Error bar* represents ±SEM. *Nml*, Normal; *C.A.*, condyloma acuminatum; *CIN*, cervical intraepithelial neoplasia; *SCCA*, squamous cell carcinoma; *EGF*, epidermal growth factor; *TFR*, transferrin factor receptors.

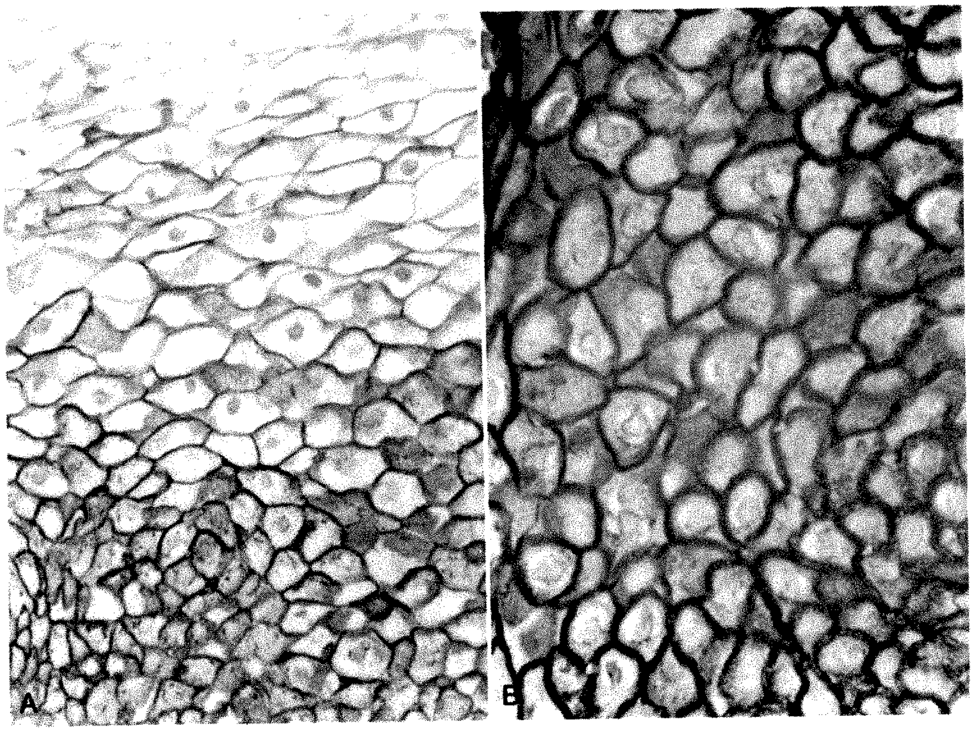

Fig. 4. Immunohistopathologic results of cervical biopsy tissue sections. **A,** Immunohistochemical staining with anti–epidermal growth factor receptor of grade 1 cervical intraepithelial neoplasia lesion showing outlining of membranous epidermal growth factor receptors in basal and focally in parabasal cell layers. (Original magnification ×325.) **B,** Anti–epidermal growth factor receptor staining of grade 3 cervical intraepithelial neoplasia lesion demonstrating intense membranous and focally granular cytoplasmic staining of full thickness of epithelium. (Original magnification ×400.)

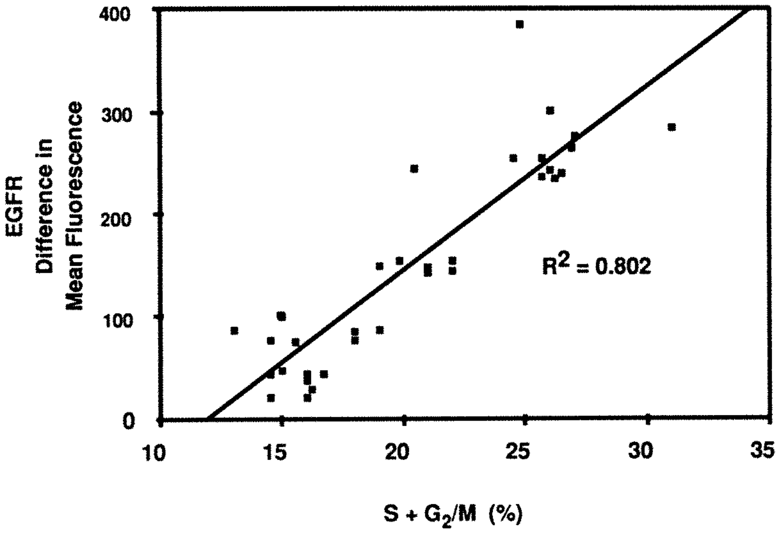

Fig. 5. Correlation of epidermal growth factor receptor expression and proliferative index by linear regression analysis. Mean fluorescence of epidermal growth factor receptor staining by flow cytometry was correlated with respective proliferative index of each sample and analyzed by simple linear regression. Analysis reveals linearity of data with high degree of correlation ($r = 0.802$). *EGFR*, Epidermal growth factor receptor.

epithelial neoplasia. Thus, although distinct differences in the intensity of staining for the epidermal growth factor receptor for varying histologic grades are possible, immunohistochemistry was relatively insensitive to the lower levels of expression of these markers that could be detected and quantified by flow cytometry.

Because of the wide range of fluorescence for the epidermal growth factor receptor in low-grade and high-grade neoplasias, it might be postulated that it was present at higher levels in dividing versus quiescent cells. Flow cytometric analysis of forward and side scatter parameters, which are measures of cellular size and internal complexity, demonstrated a uniform pattern for entire populations of cells. As seen in Table II, the proportion of cells in the proliferative phases of the cell cycle for squamous cell carcinoma is 1.6 times that of normal cells, while the expresison of epidermal growth factor receptor is increased sevenfold. Although no attempt was made to simultaneously analyze samples for epidermal growth factor receptor expression and proliferative index, a regression analysis of these two parameters was performed on data from the individual samples; this is illustrated in Fig. 5. The correlation of epidermal growth factor receptor and the proportion of S + G_2/M phase cells was found to be linear with a high level of significance ($r = 0.802$). Thus the compilation of these data suggests that epidermal growth factor receptor is correlated with both papillomavirus infection and severity of histologic grade and that biologically this marker is related to a increased proliferative index.

Comment

Autocrine and paracrine stimulation of cell surface receptors by growth factors is one mechanism by which epithelial cells are maintained in a state of cell division. Epidermal growth factor and transferrin factor are two cytokines important to the proliferation of normal keratinocytes and ectocervical cells, whereby the expression of the cell surface receptors for these factors is correlated with the level of cellular proliferation.[13] Similarly, cytoplasmic vesicles containing IL-1 are present in high levels in rapidly dividing epidermal tissue in certain disease states, and IL-1 by itself can stimulate cellular division in an autocrine manner.[22] To study the role of these selected markers of proliferation, we have used flow cytometric measurement of these factors on samples of exfoliated cervical cells from a cohort of patients with normal, dysplastic, and malignant cervical lesions, as well as continuous cell lines containing HPV DNA. Flow cytometric analysis offers a greater degree of sensitivity and a quantitative measure for these membrane and cytoplasmic proteins not available with conventional histochemical methods. In combinaton with DNA hybridization and DNA cell-cycle analysis, we have been able to uniquely examine several parameters of cellular growth and differentiation in a series of normal and HPV-associated cervical lesions.

Flow cytometry is a highly sensitive technique for the simultaneous analysis of a population of cells for membrane and cytoplasmic proteins, as well as for the analysis of the cell cycle. Cytoplasmic proteins such as filaggrin and cytokeratin are markers of cellular differentiation and have been shown to be predictive of the neoplastic conversion of cervical epithelium.[1] In this study low-molecular-weight cytokeratin was strongly expressed in a series of samples of cervical cells showing high histologic grade and was correlated with the HPV DNA of types 16, 18, and 31, whereas filaggrin was detected in normal cervices or low-grade cervical lesions. The evaluation of the full spectrum of expression of the cytokeratins and other related intermediate filaments in cervical tissues has been reported, and these studies concur with our own that alteration of the cytoskeletal filaments is a component of the neoplastic process.

The proliferative activity of a population of cells is related to the level of the expression of cytokines or their receptors and may also be one of the mechanisms by which papillomavirus gene products modify cellular processes leading to neoplastic transformation. Transferrin factor receptor is a ubiquitous marker of cell proliferation and one of the earliest signals in subsequent events leading to cell mitosis.[23] The expression of transferrin factor receptor was increased slightly in samples from high-grade dysplasias infected with papillomaviruses. Similarly, the expression of IL-1, which stimulates epidermal cell growth in an autocrine manner in certain disease states,[22] increased slightly over the spectrum of normal and HPV-infected cervical tissues.

Epidermal growth factor receptor was differentially expressed in cervical tissue, increasing with high histologic grade, and associated with a higher proliferative index. Increased expression of epidermal growth factor receptor expression on the cell surface is known to occur in several epithelial neoplasms.[24] A truncated form of the epidermal growth factor receptor has been described and shown to be expressed at high levels in cell lines derived from a squamous cell carcinoma of the vulva and in certain breast and kidney tumors.[12] This form of the epidermal growth factor receptor is neither transcribed to a greater amount nor more resistant to degradation but rather has been shown to be defective in its ability to be internalized and recycled to the cell surface after the binding of epidermal growth factor. A relationship between epidermal growth factor receptor expression and HPV infection has been reported in a select group of mucosal and cutaneous lesions.[13] Our data suggest that by using flow cytometry we can increase the sensitivity of the measurement of the epidermal growth factor receptor and to a lesser degree transferrin factor receptor and IL-1, we have found its measurement to vary significantly over the complete spectrum of HPV-associated lesions of cervical epithelium, including squamous cell carcinoma. Recently, it has been demonstrated that the E5 open reading frame of bovine papillomavirus type 1, which encodes a small hydrophobic protein with potent transforming activity in cultured rat fibroblasts, interacts with the epidermal growth factor receptor and other growth factor receptors in some way that leads to the constitutive phosphorylation of receptor proteins and inhibits its normal down regulation.[25] The observation that epidermal growth factor receptors in human cervical samples are similary expressed to higher levels in premalignant and malignant cells demonstrates that a similar process is at play in naturally arising HPV-associated lesions.

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Hemostatic wrapping of ruptured liver in two postpartum patients

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A variety of surgical techniques with variable success rates have been reported in the management of the spontaneously ruptured liver in pregnancy. We managed two cases of postpartum ruptured liver by wrapping the liver in hemostatic material. Hemostatic encapsulation effectively controlled massive bleeding of ruptured livers in two postpartum patients. (Am J Obstet Gynecol 1991;165:705-7.)

Key words: Ruptured liver, preeclampsia, hemostatic wrapping

Spontaneous rupture of the liver occurs estimately in 0.4 per 100,000 pregnancies and is associated with high maternal and fetal mortality and morbidity.[1, 2] In recent years prompt diagnosis and surgical intervention and intensive supportive measures have improved the survival rate of pregnant patients with ruptured livers. In a review by Smith et al.,[2] a variety of surgical techniques for management of spontaneously ruptured liver in pregnancy were reported. In two consecutive patients 6 years apart, with spontaneously ruptured livers in the postpartum period, we used similar hemostatic wrapping techniques to control massive bleedings. We searched the English-language literature of the past 25 years through MEDLINE Computer program with the use of a variety of relevant key words and did not find any reports on hemostatic wrapping of ruptured liver during pregnancy or the postpartum period.

Case reports

Case 1. A 24-year-old primigravid woman was first seen at term gestation with signs and symptoms of severe preeclampsia including nausea, vomiting, and right upper-quadrant pain. Pregnancy was terminated with an uneventful cesarean section. Hypovolemic shock developed 30 minutes after the operation. At laparotomy 2500 ml of fresh blood and blood clots were found in the abdomen. A 6 cm hemorrhaging laceration along the inferior anterior aspect of the right lobe and two subcapsular hematomas of the right and the

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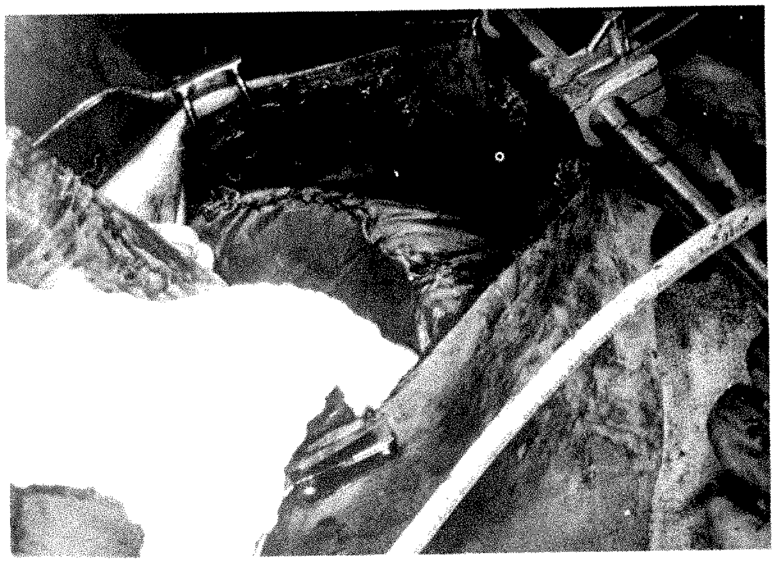

Fig. 1. The ruptured liver was released from its ligamentous attachments and encapsulated in a sheet of Vicryl suture mesh.

left lobes were initially treated with conventional surgical techniques including Pringle's maneuver, suturing and packing of the bleeding points, and later in a second laparotomy by right hepatic artery ligation. However, bleeding from the liver resumed, and after arteriography the patient was returned to the operating room. A latticework or net was fashioned from 5 mm Mersilene strip ligatures. Both hepatic lobes were completely mobilized from their diaphragmatic and peritoneal attachments and the net was snugly fitted and tied around the liver, which had been covered by strips of Surgicel gauze. Another laparotomy was done 24 hours later to control subsequent bleeding from the right adrenal gland; the liver was no longer bleeding. The postoperative course was complicated by disseminated coagulopathy, respiratory distress syndrome, prolonged hospitalization, and hepatitis B. Overall, the patient received 120 units of whole blood and blood products. At the time of a cholecystectomy 2 years later, the gross liver structure appeared to be normal. The Mersilene strip ligature net was intact with few adhesions.

Case 2. A 34-year-old, gravida 4, para 4 woman had spontaneous rupture of the liver 24 hours after delivery of an apparently uncomplicated term pregnancy. Laparotomy revealed a large amount of fresh blood and blood clots in the abdomen. The liver was grossly edematous and had hematomas and hemorrhaging tears on the anterior aspects of the right and left lobes. The liver was mobilized from its ligamentous attachments. The left lobe was wrapped snugly in a large sheet of Vicryl suture mesh. A larger sheet was fashioned by sewing two pieces of Vicryl suture mesh together, which was then wrapped around the right lobe. The sheets were sewn to each other and around the entire liver, leaving outlets for hepatic veins and porta hepatis (Fig. 1). The gall bladder was removed for ease of operation. The postoperative course was complicated by bilateral pleural effusion, transient paralytic ileus, and readmission for right pleural effusion. Two weeks after the operation, a computerized tomography scan showed a stable hematoma surrounding the anterior aspects of both lobes of the liver.

Comment

Disastrous outcome of the untreated spontaneous rupture of the liver in pregnancy has prompted introduction of a variety of classic and novel surgical management techniques.[2] Encapsulation of trauma-related, ruptured solid organs has been previously reported in surgical annals. However, we have not found any reports in English-language literature of the past 25 years on hemostatic wrapping of a ruptured liver during pregnancy or the postpartum period. We used hemostatic encapsulation of the ruptured liver in two postpartum patients, and found the technique to be a relatively easy and very effective operative method. The first patient had preeclampsia and disseminated coagulopathy, and did not respond to a variety of conventional surgical techniques. Hemostatic wrapping successfully stopped the bleeding from multiple hematoma and rupture sites of both hepatic lobes. With experience gained from the first case, the second patient with ruptured liver was expediently managed by hemostatic wrapping of the ruptured liver in sheets of absorbable Vicryl suture mesh. Elective cholecystectomy added to the ease of the operation. We believe that hemostatic wrapping can effectively control the bleeding from a spontaneously ruptured liver in postpartum patients, and may be considered as the first line of surgical management in such patients. Further experience is needed to clarify the usefulness of the method.

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Relationship between fetal biophysical activities and umbilical cord blood gas values

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In a prospective study of 62 patients undergoing cesarean section before the onset of labor a fetal biophysical profile assessment was performed within 3 hours before the cesarean section. The presence or absence of the individual fetal biophysical activities (fetal heart rate reactivity, fetal breathing movements, fetal body movements, and fetal tone) were correlated with umbilical cord blood gas and acid-base measurements (artery and vein). Fetuses with nonreactive nonstress test results or the absence of breathing had significantly lower cord artery pH, Po_2 bicarbonate, and base excess measurements but not a significantly different Pco_2 level as compared with fetuses that had these activities present. Fetuses with the absence of movements or tone had lower pH, Po_2 bicarbonate, and base excess levels and higher Pco_2 levels as compared with fetuses with the presence of movements or tone, respectively. These blood gas and acid-base differences were observed in both umbilical cord artery and vein. Subsequent analysis of the blood gas and acid-base measurements of the fetuses with compromised biophysical activities revealed that there are different levels of acidemia, hypoxemia, and hypercapnia at which the individual biophysical activities are compromised. These data suggest that the first manifestations of fetal hypoxemia and acidemia are nonreactive nonstress test results and loss of fetal breathing; in advanced acidemia, hypoxemia, and hypercapnia fetal movements and fetal tone are compromised. (AM J OBSTET GYNECOL 1991;165:707-13.)

Key words: Fetal biophysical activities, cord blood gas values

Previous studies from our institution have suggested that there is a significant relationship between fetal biophysical assessment and fetal acidemia as determined by umbilical cord pH value at the time of testing.[1] These studies, which used only cord blood pH measurements, also suggested that the first manifestations of fetal acidemia are nonreactive nonstress test (NST) results and loss of fetal breathing, whereas in advanced acidemia fetal movements and fetal tone are compromised. However, the relationships between fetal biophysical assessment and the remaining blood gas and acid-base measurements, including Po_2, Pco_2, bicarbonate, and base excess, have not been investigated in the human fetus.

This prospective study was therefore undertaken with the following objectives: (1) to investigate the relationship between the presence or absence of the in-

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Table I. Umbilical cord blood gas and acid-base measurements with reactive versus nonreactive NST results

	Reactive NST (N = 32)	*Nonreactive NST (N = 30)*	p *Value*
Artery			
pH	7.29 ± 0.04	7.21 ± 0.10	0.0007
Po_2 (mm Hg)	15.1 ± 4.8	11.9 ± 7.2	0.04
Pco_2 (mm Hg)	52.6 ± 10.4	57.7 ± 11.8	NS
Hco_3 (mmol/L)	26.1 ± 2.7	23.3 ± 3.7	0.001
Base excess (mmol/L)	−1 ± 2.3	−4.9 ± 4.8	0.0002
Vein			
pH	7.34 ± 0.04	7.27 ± 0.09	0.0002
Po_2 (mm Hg)	26.1 ± 7.9	19.9 ± 8	0.003
Pco_2 (mm Hg)	45.5 ± 7.3	49.5 ± 9.7	NS
Hco_3 (mmol/L)	25.2 ± 2.2	23 ± 3.1	0.002
Base excess (mmol/L)	−1 ± 2	−3 ± 4.1	0.01

Measurements are expressed as mean ± SD.

Table II. Umbilical cord blood gas and acid-base measurements with presence of versus absence of fetal breathing movements

	FBM present (N = 38)	*FBM absent (N = 24)*	p *Value*
Artery			
pH	7.27 ± 0.05	7.21 ± 0.12	0.003
Po_2 (mm Hg)	14.7 ± 6.2	11.5 ± 6.2	0.04
Pco_2 (mm Hg)	53.9 ± 10.9	57.3 ± 11.9	NS
Hco_3 (mmol/L)	25.7 ± 2.8	23.1 ± 3.9	0.004
Base excess (mmol/L)	−1.6 ± 2.3	−5.2 ± 5.6	0.001
Vein			
pH	7.32 ± 0.04	7.27 ± 0.10	0.007
Po_2 (mm Hg)	24.7 ± 8.2	20 ± 8.4	0.03
Pco_2 (mm Hg)	24.7 ± 8.2	50.3 ± 10.5	0.05
Hco_3 (mmol/L)	45.9 ± 7.2	23.6 ± 3.9	NS
Base excess (mmol/L)	24.3 ± 2.1	−2.8 ± 4.7	NS
	−1.5 ± 2.1		

FBM, Fetal breathing movements. Measurements are expressed as mean ± SD.

dividual fetal biophysical activites and umbilical cord blood gas measurements (pH, Po_2, Pco_2, bicarbonate, and base excess) in both artery and vein, and (2) to determine the different levels of pH, Po_2, Pco_2, bicarbonate, and base excess at which the individual biophysical activities become compromised.

Material and methods

This prospective study included 62 consecutive patients with singleton pregnancies, gestational ages between 25 and 37 weeks, and delivery by cesarean section before the onset of labor. Infants with congenital anomalies were excluded. In all cases fetal biophysical profile assessment and umbilical artery velocimetry were performed within 3 hours before the cesarean section. The relationship between fetal biophysical assessment, umbilical artery velocimetry, and fetal acidemia in this group of patients was investigated and is reported separately.[2] The primary indications for delivery were severe pregnancy-induced hypertension (34 patients, 55%), severe pregnancy-induced hypertension associated with intrauterine growth retardation (14 patients, 22%), severe intrauterine growth retardation (8 patients, 13%), and placenta previa (6 patients, 10%). All patients had intact membranes and all received epidural analgesia. All cesarean sections were performed with the patient in the left lateral position. Epidural analgesia was achieved with 0.5% bupivacaine hydrochloride after infusion of 1000 ml lactated Ringer's injection solution. Nasal oxygen was administered 3 L/min from the time satisfactory blockade was achieved until delivery of the infant. None of the patients was hypotensive.

The fetal biophysical assessment was performed according to a previously described method with a maximum period of observation of 30 minutes.[3] For the purpose of this study, a nonreactive NST result was defined as one or fewer fetal heart rate (FHR) accelerations (15 beats/min for 15 seconds) in ≥ 40-minute period. Absence of fetal breathing was defined as the absence of at least one epsiode of fetal breathing lasting 30 seconds within 30 minutes. Absence of fetal

Table III. Umbilical cord blood gas and acid-base measurements with presence of versus absence of fetal body movements

	FM present (*N = 53*)	*FM absent* (*N = 9*)	p *Value*
Artery			
pH	7.27 ± 0.06	7.08 ± 0.10	0.0001
Po_2 (mm Hg)	14.2 ± 6.3	8.3 ± 4.1	0.01
Pco_2 (mm Hg)	53.1 ± 10	69.3 ± 10	0.0001
Hco_3 (mmol/L)	25.2 ± 3.2	20.9 ± 3.6	0.001
Base excess (mmol/L)	−2 ± 3	−10 ± 4.9	0.0001
Vein			
pH	7.32 ± 0.06	7.17 ± 0.07	0.0001
Po_2 (mm Hg)	23.8 ± 8.5	16.6 ± 5.9	0.02
Pco_2 (mm Hg)	46.5 ± 7.2	55.1 ± 14.3	0.009
Hco_3 (mmol/L)	24.5 ± 2.4	21 ± 4.5	0.001
Base excess (mmol/L)	−1.2 ± 2.6	−7 ± 3.6	0.0001

FM, Fetal body movements. Measurements are expressed as mean ± SD.

Table IV. Umbilical cord blood gas and acid-base measurements with presence of versus absence of fetal tone

	FT present (*N = 57*)	*FT absent* (*N = 5*)	p *Value*
Artery			
pH	7.27 ± 0.06	7.03 ± 0.04	0.0001
Po_2 (mm Hg)	14.1 ± 6.2	6.2 ± 2.7	0.007
Pco_2 (mm Hg)	53.7 ± 10	72 ± 12.6	0.0003
Hco_3 (mmol/L)	25.1 ± 3.3	20.2 ± 2	0.002
Base excess (mmol/L)	−2.2 ± 3.4	−12.1 ± 2.5	0.0001
Vein			
pH	7.31 ± 0.06	7.14 ± 0.06	0.0001
Po_2 (mm Hg)	23.7 ± 8.2	13.6 ± 7.2	0.009
Pco_2 (mm Hg)	46.7 ± 7.1	57.2 ± 18.6	0.01
Hco_3 (mmol/L)	24.3 ± 2.5	20.9 ± 5.1	0.01
Base excess (mmol/L)	−1.5 ± 2.9	−7.8 ± 3.3	0.0001

FT, Fetal tone. Measurements are expressed as mean ± SD.

body movements was defined as the presence of two or fewer fetal body movements within 30 minutes. Absence of fetal tone was defined as fetal extremities in extension without return to flexion after fetal body movements.

Immediately after delivery, a segment of the umbilical cord was doubly clamped and blood was collected separately from the artery and vein into plastic heparinized syringes. It was then transported on ice for acid-base analysis, which was done by means of an automatic pH and blood gas analyzer (model 178, Corning Medical, Boston). All pH measurements were performed within 5 minutes after the blood collection and all patients had samples drawn from both artery and vein. The pH, Po_2, and Pco_2 were directly measured whereas the bicarbonate and base excess levels were calculated. The comparison of blood gas values between fetuses with and without the presence of biophysical activites was performed with the Student *t* test. A one-tail *t* test was used to check for existing differences of the various blood gas levels at which the biophysical activities are compromised. A *p* value < 0.05 was considered statistically significant.

Results

The mean (±SD) gestational age was 31.9 (±3.1) weeks. On the basis of fetal biophsyical assessment before the cesarean section, 30 fetuses had nonreactive NST results, 24 had absence of breathing, nine had absence of body movements, and five had absence of tone. Tables I to IV compare the blood gas and acid-base measurements between fetuses with and without the presence of biophysical activities. As shown in Table I, fetuses with nonreactive NST results had significantly lower pH, Po_2, bicarbonate, and base excess levels as compared with fetuses with reactive NST results. There was no difference in the Pco_2 level between these two groups of fetuses. The same relationships were noted in both cord artery and vein measurements. As shown in Table II, fetuses with the absence of breathing also had lower levels of cord artery pH, Po_2, bicarbonate, and base excess but not significantly different Pco_2 levels as compared with fetuses with breathing present. Tables V and VI show the blood gas values of both the artery and vein at which the biophysical activities become compromised.

Figs. 1 to 5 illustrate the comparison of the cord

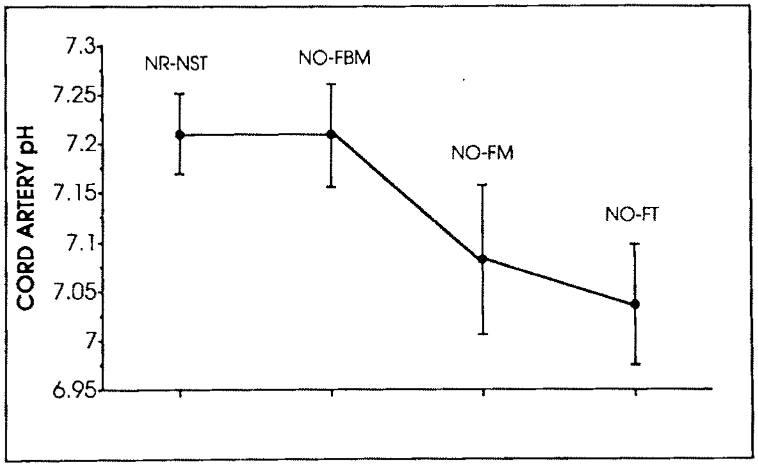

Fig. 1. Relationship beetween cord artery pH and absence of fetal biophysical activities. The pH tends to be lower in the absence of body movements or tone as compared with nonreactive NST results or absence of breathing. Results are expressed in means ± 95% error bars. *NR-NST*, Nonreactive NST results; *NO-FBM*, absence of fetal breathing; *NO-FM*, absence of fetal movements; *NO-FT*, absence of fetal tone.

Table V. Umbilical artery blood gas values associated with compromised biophysical activities

	No. of fetuses	*pH*	*P_{O_2} (mm Hg)*	*P_{CO_2} (mm Hg)*	*Bicarbonate (mmol/L)*	*Base excess (mmol/L)*
1. Nonreactive NST	30	7.21 ± 0.10	11.9 ± 7.2	57.7 ± 11.8	23.3 ± 3.7	−4.9 ± 4.8
2. Absence of FBM	24	7.21 ± 0.12	11.5 ± 6.2	57.3 ± 11.9	23.1 ± 3.9	−5.2 ± 5.6
3. Absence of FM	9	7.08 ± 0.10	8.3 ± 4.1	69.3 ± 10.0	20.9 ± 3.6	−10.0 ± 4.9
4. Absence of FT	5	7.03 ± 0.04	6.2 ± 2.7	72.0 ± 12.6	20.2 ± 2.0	−12.1 ± 2.5

NST, Nonstress test; *FBM*, fetal breathing movements; *FM*, fetal body movements; *FT*, fetal tone. Results are expressed as means with 1 SD. One-tailed Student's *t* test: for pH differences, $p < 0.05$: 1 vs 3; 1 vs 4; 2 vs 3; 3 vs 4; for P_{O_2} differences, $p < 0.05$: 1 vs 4; 2 vs 4; for P_{CO_2} differences, $p < 0.05$: 1 vs 3; 1 vs 4; 2 vs 3; 3 vs 4; for bicarbonate differences, $p < 0.05$: 1 vs 3; 1 vs 4; for base excess differences, $p < 0.05$: 1 vs 3; 1 vs 4; 2 vs 3, 3 vs 4.

artery blood gas and acid-base measurements associated with the absence of biophysical activities. Although no differences were found between nonreactive NST results and the absence of fetal breathing, significant differences were found between either nonreactive NST results or the absence of fetal breathing versus the absence of fetal body movement or tone, as well as between absent fetal movement versus absent tone. The absence of fetal movements or tone was associated with lower pH, P_{O_2}, bicarbonate, and base excess levels and higher P_{CO_2} levels as compared with the absence of breathing or nonreactive NST results. Similar relationships were noted in both artery and vein measurements.

Comment

The purpose of antepartum fetal biophysical assessment is to avoid fetal death in utero and perhaps some hypoxic complications of the neonate related to intrauterine asphyxia. Because fetal asphyxia is the common end pathway to fetal death in many high-risk pregnancies, it is reasonable to investigate the ability of the individual biophysical components to detect the different degrees of fetal hypoxemia, hypercapnia, and acidemia. Although the relationship between fetal acidemia, as determined by cord blood pH measurements, and fetal biophysical assessment has been previously reported by our group,[1] we are not aware of data regarding the different levels of fetal hypoxemia or hypercapnia at which the biophysical activities become compromised. The purpose of this investigation was to investigate the relationship of the individual biophysical components and fetal hypoxemia, hypercapnia, and acidemia as determined by umbilical cord blood gas and acid-base measurements. In our previous study[1] we examined only the relationship between biophysical assessment and cord blood pH value. With this study we extend our observations to the relationship between the individual biophysical components and the remaining blood gas values (P_{O_2}, P_{CO_2}, bicarbonate, and base excess). This study design was such that all patients re-

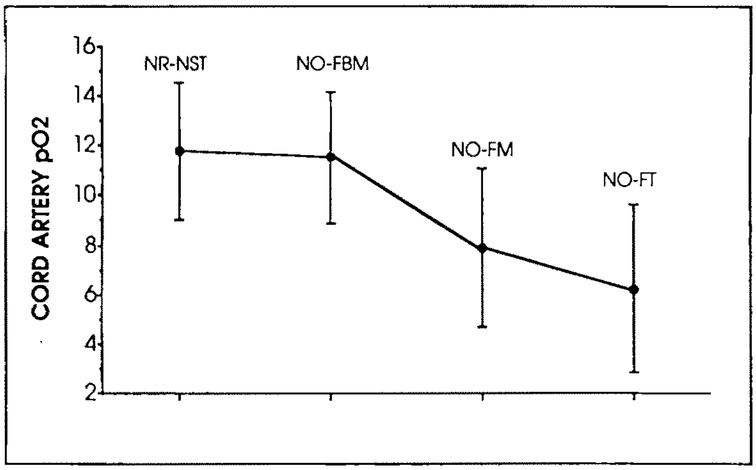

Fig. 2. Relationship between cord artery Po_2 and absence of fetal biophysical activities. The Po_2 tends to be lower in the absence of body movements or tone as compared with nonreactive NST results or absence of breathing. Results are expressed in millimeters of mercury (means ± 95% error bars). *NR-NST*, Nonreactive NST results; *NO-FBM*, absence of fetal breathing; *NO-FM*, absence of fetal movements; *NO-FT*, absence of fetal tone.

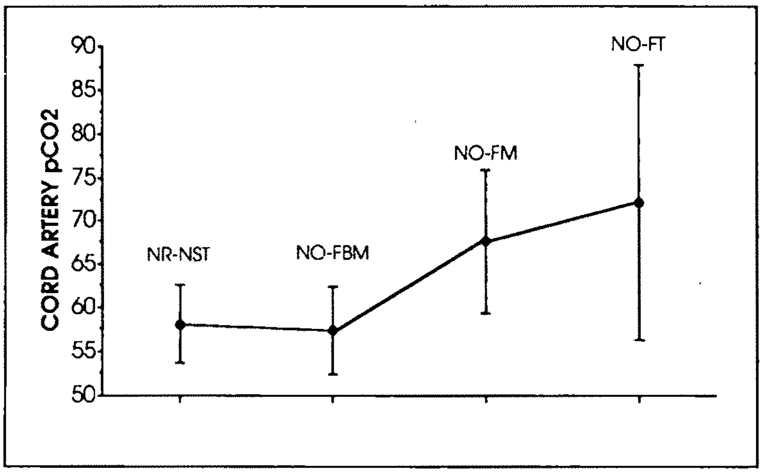

Fig. 3. Relationship between cord artery Pco_2 values and absence of fetal biophysical activities. The Pco_2 tends to be higher in the absence of body movements or tone as compared with nonreactive NST results or absence of breathing. Results are expressed in millimeters of mercury (means ± 95% error bars). *NR-NST*, Nonreactive NST results; *NO-FBM*, absence of fetal breathing; *NO-FM*, absence of fetal movements; *NO-FT*, absence of fetal tone.

Table VI. Umbilical artery blood gas values associated with compromised biophysical activities

	No. of fetuses	*pH*	Po_2 *(mm Hg)*	Pco_2 *(mm Hg)*	*Bicarbonate (mmol/L)*	*Base excess (mmol/L)*
1. Nonreactive NST	30	7.27 ± 0.09	19.9 ± 8.0	49.5 ± 9.7	23.0 ± 3.1	−3.0 ± 4.1
2. Absence of FBM	24	7.27 ± 0.10	20.0 ± 8.4	50.3 ± 10.5	23.6 ± 3.9	−2.8 ± 4.7
3. Absence of FM	9	7.17 ± 0.07	16.6 ± 5.9	55.1 ± 14.3	21.0 ± 4.5	−7.0 ± 3.6
4. Absence of FT	5	7.14 ± 0.06	13.6 ± 7.2	57.2 ± 18.6	20.9 ± 5.1	−7.8 ± 3.3

NST, Nonstress test; *FBM*, fetal breathing movements; *FM*, fetal body movements; *FT*, fetal tone. Results are expressed as means with 1 SD. One-tailed Student's t test: for pH differences, $p < 0.05$: 1 vs 3; 1 vs 4; 2 vs 3; 3 vs 4; for Po_2 differences, $p < 0.05$: 1 vs 4; for bicarbonate differences, $p < 0.05$: 2 vs 3; for base excess differences, $p < 0.05$: 1 vs 3; 1 vs 4; 2 vs 3; 3 vs 4.

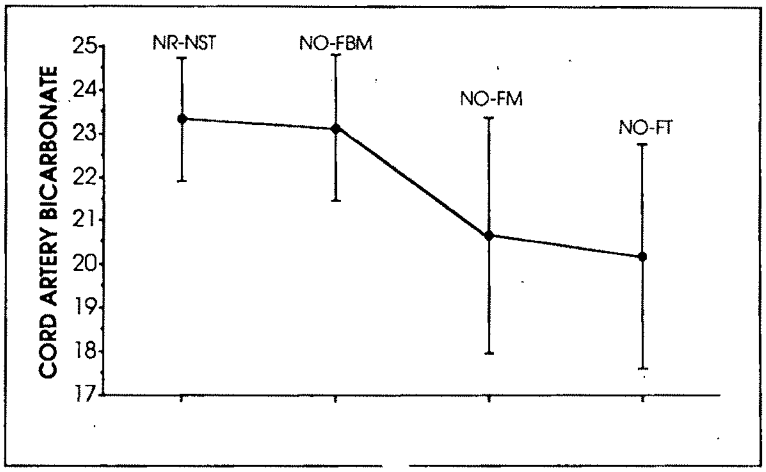

Fig. 4. Relationship between cord artery bicarbonate and absence of fetal biophysical activities. The bicarbonate tends to be lower in the absence of body movements or tone as compared with nonreactive NST results or absence of breathing. Results are expressed in millimoles per liter (means ± 95% error bars). *NR-NST*, Nonreactive NST results; *NO-FBM*, absence of fetal breathing; *NO-FM*, absence of fetal movements; *NO-FT*, absence of fetal tone.

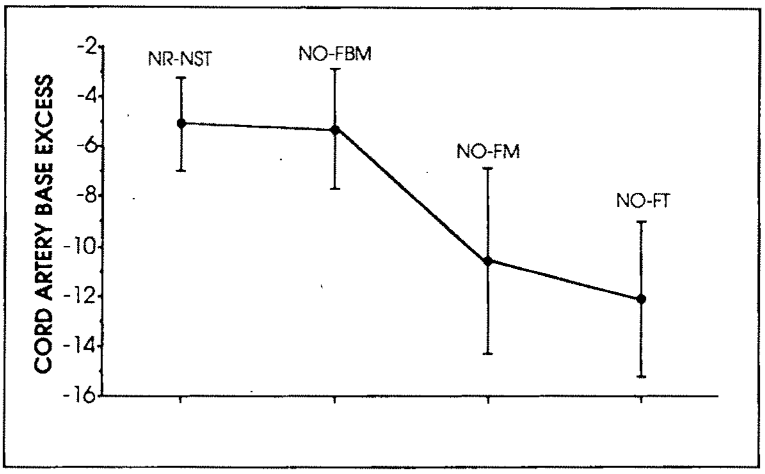

Fig. 5. Relationship between cord artery base excess and absence of fetal biophysical activities. Base excess tends to be lower in the absence of body movements or tone as compared with nonreactive NST results or absence of breathing. Results are expressed in millimoles per liter (means ± 95% error bars). *NR-NST*, Nonreactive NST results; *NO-FBM*, absence of fetal breathing; *NO-FM*, absence of fetal movements; *NO-FT*, absence of fetal tone.

ceived the same technique to induce anesthesia (epidural) so that comparisons of the blood gas values between the different groups were possible and appropriate.

These data suggest that the acute markers of the fetal conditions (FHR reactivity, fetal breathing movements, fetal body movements, and fetal tone) are strongly correlated with fetal acid-base status at the time of testing including not only pH but also most of the other acid-base parameters. The presence of FHR reactivity (reactive NST results) and the presence of fetal breathing were always associated with cord blood pH values greater than 7.20. As shown in Tables I to IV, the presence or absence of the acute markers of fetal condition is related to fetal acid-base status at the time of testing. The absence of FHR reactivity (nonreactive NST results) and the absence of fetal breathing were associated with lower levels of pH, Po_2, bicarbonate, and base excess as compared with the presence of these activities. However, we found no significant differences in the Pco_2 levels between reactive and nonreactive NST results or between presence or absence of fetal breathing. Absence of fetal body movements or tone was associated with significantly lower pH, Po_2, bicarbonate, and base excess levels and higher P_{CO_2} levels as compared with the presence of these activities. These observations, in combination with the much lower pH and P_{O_2} levels that were observed in fetuses with the absence of movements and tone, would strongly suggest that hypercapnia is a relatively late event in the development of antepartum intrauterine asphyxia. These data suggest that the first clinical manifestations of fetal hypoxemia and acidemia are nonreactive NST results and loss of fetal breathing. In advanced acidemia, hypoxemia, and hypercapnia, fetal body movements and fetal tone are compromised.

Our previous study[1] has provided information regarding the pH level below which the fetal central nervous system centers malfunction. Our previous data have suggested that the FHR reactivity and fetal breathing centers cease when the pH is lower than 7.20; the centers controlling body movement and tone begin to malfunction at pH values of 7.10 to 7.20 and are completely abolished at a pH level lower than 7.10. This study extends our observations and establishes the levels of all other remaining acid-base parameters including pH, P_{O_2}, P_{CO_2}, and base excess, at which the biophysical activities become compromised (Tables V and VI). Our present observations support the concept of different levels of sensitivity not only to acidemia but also to hypoxemia and hypercapnia. The concept of nonreactive NST results and the absence of fetal breathing being one of the first manifestations of fetal hypoxemia and acidemia, whereas the absence of fetal body movements and tone indicate the presence of advanced acidemia, hypoxemia, and hypercapnia is very important for correct interpretation and application of the information obtained from fetal biophysical monitoring.

Amniotic fluid volume, unlike the fetal biophysical activities, is not influenced acutely by alterations in fetal acid-base status and represents a chronic marker of the fetal condition. We still include amniotic fluid assessment in the fetal biophysical profile because of the high correlation between decreased amniotic fluid volume and increased incidence of abnormal pregnancy outcome.[3, 4]

These data suggest that sequential assessment of the individual biophysical components makes sense as we have proposed in the past.[5] The different sensitivity levels of the individual biophysical activities apply not only to different degrees of acidemia but also to different degrees of hypoxemia and hypercapnia. In the presence of reactive NST results or fetal breathing, there is nothing to be gained by also assessing body movements and tone. On the other hand, in the absence of FHR reactivity and breathing it is important to assess the presence of body movements and tone because if these activities are also absent then one should suspect advanced acidemia, hypoxemia, and hypercapnia. The understanding of the physiology and pathophysiology of the fetal biophysical activities is essential for estimating the degree of fetal compromise and a prerequisite for the appropriate clinical applicaton of fetal biophysical assessment.

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Prenatal diagnosis of fetal cytomegalovirus infection

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Twelve fetuses were evaluated with a combination of ultrasonography, amniocentesis, and blood sampling for possible cytomegalovirus infection. In seven the mother had a documented primary cytomegalovirus infection. All seven women had normal ultrasonographic findings and one fetus was found to be infected. In the other five cases fetal cytomegalovirus infection was diagnosed in association with abnormal ultrasonographic findings. There was no history of maternal infection in the latter group. All positive and negative diagnoses were confirmed and none of the six infected fetuses survived. In this series, the most reliable parameters of infection were the isolation of the virus from amniotic fluid and elevations of total immunoglobulin M and γ-glutamyl transpeptidase in fetal blood. In the majority of infected fetuses cytomegalovirus-specific immunoglobulin M was not detected in blood. Prenatal diagnosis of fetal cytomegalovirus infection is possible with a combination of amniocentesis and fetal blood sampling. (AM J OBSTET GYNECOL 1991;165:714-8.)

Key words: Cytomegalovirus, prenatal diagnosis, fetus

Cytomegalovirus is the most common cause of intrauterine infection, affecting an estimated 1% of all live births in the United States.[1] Fetal infection may follow either primary or reactivated maternal infection, although symptomatic congenital disease is more likely to occur in association with primary maternal infection.[2] A total of 30% to 40% of pregnant women with primary cytomegalovirus infection transmit the virus to their fetuses.[3] Although only 10% of those infected are symptomatic at birth, 20% to 30% die and late complications almost invariably develop among the survivors. In addition, long-term neurologic sequelae develop later in childhood in 5% to 15% of congenitally infected infants without symptoms.[4]

The natural history of intrauterine cytomegalovirus infection is not well understood, but it is clear that some fetuses are irreversibly damaged by the virus before delivery. Those infants would not derive much benefit from postnatal therapy, but if infected fetuses could be detected before this irreversible stage has been reached, treatment in utero might have a significant effect on the course of the disease. The recent development of effective antiviral agents with in vitro and in vivo activity against human cytomegalovirus may make such therapy possible.[5] Daffos et al.[6] have shown that in utero treatment of fetuses infected with toxoplasmosis significantly improves the outcome in those cases. However, before consideration can be given to treatment of cytomegalovirus in utero, it is necessary to be able to accurately diagnose fetal infection with this organism. In addition to traditional fibroblast cultures, new methods including the use of monoclonal antibodies, deoxyribonucleic acid (DNA)–ribonucleic acid (RNA) hybridization and DNA amplification by the polymerase chain reaction have been applied to the rapid diagnosis of cytomegalovirus infection. The sensitivity and specificity of these methods in the detection of fetal infection remains to be determined. This article describes our experience with the prenatal diagnosis of cytomegalovirus infection in 12 fetuses with either exposure to documented primary maternal infection during pregnancy or abnormal ultrasonographic results suggestive of fetal cytomegalovirus infection.

Material and methods

Patients at risk of fetal cytomegalovirus infection were evaluated at the Mount Sinai Medical Center or the Institut de Puericulture de Paris between 1982 and 1989. Seven women were seen because of documented primary maternal cytomegalovirus infection, and five additional patients were investigated because of abnormal ultrasonographic findings suggestive of fetal infection. All patients underwent detailed ultrasonographic examinations and 11 of the 12 also had percutaneous fetal blood sampling and simultaneous amniocentesis. Amniotic fluid was cultured for cytomegalovirus. Complete blood cell count, γ-glutamyl

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transpeptidase (GGTP), total immunoglobulin M (IgM), cytomegalovirus-specific IgM, and cytomegalovirus cultures were performed on the fetal blood samples. In addition, polymerase chain reaction with cytomegalovirus-specific oligonucleotides was performed on two amniotic fluid and fetal blood samples obtained at the Mount Sinai Medical Center.

All cytomegalovirus cultures, specific IgM assays, and polymerase chain reaction studies of those fetuses evaluated at the Mount Sinai Medical Center were performed in the laboratory of one of the authors (D.E.) at Memorial Sloan-Kettering Cancer Center. Cytomegalovirus-specific IgM was determined by enzyme-linked immunosorbent assay (ELISA). Cytomegalovirus isolation was performed in fibroblast culture by indirect immunofluorescence staining with cytomegalovirus-specific monoclonal antibodies. Results were generally available within 48 hours after innoculation. The polymerase chain reaction studies on amniotic fluid and fetal blood were performed by previously described techniques.[7] We used oligonucleotide primers flanking a 421 base pair sequence of the cytomegalovirus immediate-early gene and an oligonucleotide probe to an internal sequence of the amplified sequence. Positive and negative controls were analyzed simultaneously with each study sample. Specific details regarding the polymerase chain reaction primers and probe and the cytomegalovirus-specific monoclonal antibodies will be furnished on request.

At the Institute of Puericulture de Paris, cytomegalovirus-specific IgM studies were also performed by ELISA. Cytomegalovirus cultures were done by fibroblast innoculation without immunofluorescence, and results were available within 3 to 4 days after innoculation.

Complete blood counts were done by standard automatized techniques, and all abnormal platelet counts were confirmed manually. Total IgM and GGTP were assayed by standard techniques at both centers. Hematocrit values, platelet counts, and total IgM values were compared with normal fetal values for the corresponding gestational age.[8] GGTP levels do not change with gestational age, and the values in this series were compared with known fetal norms (24.4 ± 9.6 UI/L).[9]

Fetal blood sampling was done percutaneously under ultrasonic guidance, and amniotic fluid was obtained immediately after the blood had been aspirated. The fetal origin and purity of the blood was confirmed in all cases by red blood cell size analysis.

Results

Primary infections. Seven pregnancies were studied because of documented primary infections in the mothers (Table I, patients 1 to 7). All these women had clinical manifestations of cytomegalovirus infection, and cytomegalovirus-specific IgM present in their serum. The infections all occurred during the first half of pregnancy (range, 11 to 18 weeks), and the patients were referred for fetal assessment between 21 and 32 weeks' gestation. One of these fetuses was found to be infected with cytomegalovirus. This case (Table I, No. 1) has been previously reported.[10] Briefly, the mother had fever, neurologic symptoms, and liver dysfunction at 11 weeks' gestation and she was shown to have cytomegalovirus-specific IgM. At 21 weeks' gestation ultrasonographic examination revealed an appropriately grown fetus and no structural abnormalities or evidence of hydrops. Fetal blood studies revealed a normal hematocrit value and platelet count, and cytomegalovirus-specific IgM was absent. The GGTP value was elevated, however, and cultures of the amniotic fluid and fetal blood were positive for cytomegalovirus. This pregnancy was terminated. Autopsy revealed a grossly normal fetus with histologic evidence of nonspecific acute infection (white cell infiltrates in the lung and liver). There were rare inclusion cells suggestive of cytomegalic inclusion disease. Immunofluorescence tissue studies were positive for cytomegalovirus in the fetal kidney, lung, adrenal, and placenta.

The six remaining fetuses of mothers with documented infection were sampled between 24 and 32 weeks' gestation. All were structurally normal on ultrasonographic examination. The fetal hematocrit, platelet count and total IgM levels were normal. Specific cytomegalovirus IgM and viral cultures of amniotic fluid and fetal blood were negative in all cases. These fetuses were delivered at term, and all except one were appropriately grown. None had manifestations of cytomegalovirus infection. Specific IgM and viral cultures were negative in all neonates.

Abnormal ultrasonographic findings. Five additional fetuses were studied because of abnormal ultrasonographic findings and all were infected. None of these five fetuses survived; three pregnancies were terminated and the two live-born infants died within a few days (Table I, patients 8 to 12). Patient No. 9 did not undergo amniocentesis or fetal blood sampling. In this instance the pregnancy was terminated because of the ultrasonographic findings and the diagnosis was made retrospectively by culturing tissues from the abortus. In all five cases cytomegalovirus infection was confirmed by isolation of the virus from fetal tissues after termination of pregnancy or in the urine of live-born infants during the first week after birth. All the mothers were cytomegalovirus IgG seropositive but none had cytomegalovirus-specific IgM. Therefore it is likely that these cases were a result of recurrent infection, although a primary infection earlier during the pregnancy cannot be ruled out. One case was confirmed to

Table I. Patient data

Patient No.	Reason for investigation	Type of infection	Maternal IgM	Gestational age at PUBS (wk)	Ultrasonographic findings	Platelet count	Fetal blood		
							GGTP	Total IgM	CMV IgM
1*	Primary CMV infection at 13 wk	Primary	+	21	Normal	N	↑	N/A	–
2-7†	Maternal infection during pregnancy	Primary	+ (all cases)	24-32	Normal anatomy, one mild growth retardation	N	N	N	–
8*	Abnormal ultrasonographic findings at 21 wk	Recurrent	–	22	Ascites, dilatation of posterior horns of lateral ventricles	↓	↑	↑	–
9*	Abnormal ultrasonographic findings at 16 wk	Unknown	–	ND	Oligohydramnios, dilatation of posterior horns of lateral ventricles, bowel echodensity	NA	NA	NA	NA
10†	Fetal growth retardation at 30 wk	Unknown	–	33	Oligohydramnios, fetal growth retardation	N	NA	↑	–
11†	Abnormal ultrasonographic findings at 31 wk	Unknown	–	31	Oligohydramnios, cerebral calcification, ventricular dilatation	N	↑	↑	+
12*	Abnormal ultrasonographic findings at 30 wk	Unknown	–	30	Severe hydrops	N	↑	↑	–

PUBS, Percutaneous umbilical blood sampling; *CMV,* cytomegalovirus; *NA,* not available; *N,* normal; *AF,* amniotic fluid; *TOP,* termination of pregnancy; +, positive; –, negative; *PCR,* polymerase chain reaction; *ND,* not done.

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†Evaluated at the Institut de Puericulture de Paris.

be a recurrence because at 10 weeks' gestation the mother was found to have cytomegalovirus-specific IgG and no IgM.

The ultrasonographic findings in this group were varied. The most common abnormalities were ventricular dilatation (three cases) and oligohydramnios (three cases). One fetus (patient No. 10) was thought to be growth retarded, and this was the only one with a birth weight below the 10th percentile. Not only were the ultrasonographic findings diverse among the patients, but in a given fetus they sometimes changed. For example, in the fetus of patient No. 8 moderate ascites at 22 weeks' gestation resolved almost completely within 1 week.

Comment

Approximately 10% of infants congenitally infected with cytomegalovirus are symptomatic at birth.[4] The most common findings are petechiae, hepatosplenomegaly, jaundice, microcephaly, small size for gestational age, inguinal hernias in males, and chorioretinitis. The mortality in this group may be as high as 30%, and 90% of the survivors have permanent disabilities such as mental retardation, neuromuscular disorders, and hearing loss. Permanent sequelae, which is usually hearing loss, develop in 5% to 15% of the infected infants without symptoms, but mental retardation and severe neuromuscular disorders may also occur.[4, 11]

Between 30% and 40% of infants exposed to primary maternal cytomegalovirus infection will be congenitally infected.[3] It is not known why most exposed fetuses do not acquire the infection. Gestational age at the time of maternal infection does not affect the transmission rate, although it does seem to influence the severity of the infection. In the report by Stagno et al.[3] of 37 infected infants, all those who were symptomatic at birth and those in whom clinically significant handicaps subsequently developed were born to women with primary infection before 27 weeks' gestation.

If 1% of all babies delivered in the United States are congenitally infected, and approximately 20% of these have permanent sequelae, it is obvious that cytomegalovirus is a significant public health problem. Surprisingly, there are only a few isolated reports regarding the prenatal diagnosis of this infection.[12] The most obvious explanation for this is that the great majority of primary cytomegalovirus infections are without

CMV cultures		*PCR*			
AF	*Blood*	*AF*	*Blood*	*Outcome*	*Autopsy findings*
+	+	NA	NA	TOP	Normal fetus; histologic evidence of infection
–	–	NA	NA	Normal infants, one small for gestational age	—
+	–	+	–	TOP	Ventricular dilatation with massive necrosis of right hemisphere; placental villitis
NA	NA	NA	NA	TOP	Disseminated CMV infection; ventricular dilatation with brain necrosis and calcifications; placental villitis
+	–	NA	NA	Delivery at 36 wk; hepatic failure; thrombocytopenia; small for gestational age; neonatal death	Disseminated CMV infection; normal brain
+	–	NA	NA	TOP, microcephaly, petechia, hepatosplenomegaly	ND
+	–	+	–	Delivery at term; pulmonary hypoplasia; neonatal death	Disseminated CMV infection; normal brain

symptoms, and pregnant women are not routinely screened for cytomegalovirus antibodies. Another problem is that there is no intrauterine treatment for this disease at this time.

In this series we evaluated seven fetuses exposed to documented primary maternal infection and found only one (14%) to be infected in utero. All these fetuses, including the infected one, had normal ultrasonographic findings. By combining viral cultures of amniotic fluid and fetal blood with assessment of specific cytomegalovirus IgM, total IgM, platelet count, and GGTP in fetal blood we were able to make a correct diagnosis in each of these cases. All negative cases were confirmed after birth and cytomegalovirus was isolated from the infected fetus after termination of that pregnancy. It must be noted, however, that given the normal morphologic findings in the infected fetus it is impossible to predict what manifestations if any may have occurred after birth in that case.

Five infected fetuses were evaluated because of abnormal ultrasonographic findings. The most common finding in this group was cerebral ventricular dilatation and oligohydramnios. However, no single abnormality was consistently seen in all fetuses. Clearly, any fetus with ventricular dilatation, microcephaly, or cerebral calcifications should be suspected of being infected. Also, fetuses with any evidence of unexplained hydrops (ascites, pleural effusions, or skin edema) should be evaluated for cytomegalovirus infection. None of the fetuses with abnormal ultrasonographic findings and cytomegalovirus infection survived; three pregnancies were terminated and the two live-born infants died within a few days. All these fetuses were found to have severe manifestations of cytomegalovirus infection including three with central nervous system abnormalities. The other two had normal central nervous system findings but died of pulmonary hypoplasia and liver failure.

Five of the six infected fetuses underwent fetal blood sampling and amniocentesis. A positive viral culture of amniotic fluid and elevated total IgM and GGTP values in fetal blood were found in all five fetuses tested. Cytomegalovirus culture of fetal blood was positive in only one of five fetuses tested. Cytomegalovirus-specific IgM was present in fetal blood in only one of the five fetuses tested, compared with rates of 50% to 90% found in cord blood of infected neonates.[13, 14] The fetal immune system has been found to be capable of consistently responding to certain antigenic stimuli by 22 weeks' gestation.[15] However, production of specific IgM appears to be dependent on the organism involved. For example, it has been found that the vast majority of fetuses with congenital rubella infection produce specific IgM after 22 weeks,[15] whereas only 15% of fetuses infected with toxoplasma tested between 24 to 29 weeks produce specific IgM.[6] The low rate of positivity in our patients may be because two of the patients were sampled at 21 and 23 weeks' gestation, or it may be related to the IgM assays used. Ahlfors et al.[13] sent serum from culture proved cytomegalovirus-infected neonates to four different laboratories for analysis. The three that used an ELISA detected cytomegalovirus-specific IgM in 63% to 80% of the cases, whereas the one with a radioimmunoassay detected positivity in only 50% of cases. Other authors[14] have reported that the radioimmunoassay is 90% sensitive. It is clear from all studies that viral cultures remain the "gold standard." In this report only ELISAs were used, but they were performed in two different laboratories. Because the number of patients is small, the true sensitivity of specific IgM analysis cannot be determined from this series, but it is evident that this parameter alone cannot be used to exclude congenital cytomegalovirus infection.

In conclusion, the prenatal diagnosis of cytomegalovirus infection is possible by use of a combination of ultrasonography, amniocentesis, and fetal blood sampling. Although amniotic fluid culture for cytomegalovirus seems to be very sensitive, the number of cases in this series is not large enough to advocate use of amniocentesis as the only diagnostic approach. Furthermore, in a review Stagno et al.[16] mentioned that they were aware of three women with primary gestational cytomegalovirus infection in whom the virus was isolated from fetal or placental tissues but not from amniotic fluid. In our opinion, until more data become available, fetal blood should also be studied when evaluating a fetus for cytomegalovirus infection. Furthermore, it is possible that fetuses with hematologic evidence of systemic involvement, such as thrombocytopenia and liver damage, may have a worse prognosis than those infected without these findings. Once the diagnosis has been made, if the fetus has abnormal ultrasonographic findings this series suggests that the prognosis for intact survival appears to be very poor. However, if the ultrasonographic findings are normal the prognosis is far from clear. Until follow-up data of such cytomegalovirus-infected fetuses are available, we will have to rely on information obtained from neonates about the natural history of these infants.

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Increased inducibility of inflammatory mediators from peripheral blood mononuclear cells of women with salpingitis

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To investigate whether immune system activation may contribute to the tissue damage observed in salpingitis, we isolated peripheral blood mononuclear cells and quantitated production of the monocyte activation products tumor necrosis factor-α, interleukin-1, and interleukin-6. Unstimulated cells from 7 of 20 women with salpingitis spontaneously released tumor necrosis factor at a concentration >2 SD above the mean value produced by cells from 29 healthy donors. Interferon gamma (200 U/ml) further induced production of tumor necrosis factor from mononuclear cells of 11 women with salpingitis. In contrast, production of tumor necrosis factor by each of 23 other patients who lacked laparoscopic or clinical evidence of salpingitis was similar to that of the controls. In a subset of women whose cells were tested for production of other monokines, three of nine women with salpingitis spontaneously released interleukin-1 but none of the others did so. Four of nine patients with salpingitis also produced interleukin-6, but none of the others did so. None of the monokines were detected in serum from any subject. The results suggest that monocytes from women with salpingitis are primed in vivo and produce inflammatory mediators under conditions where monocytes from other women are poorly responsive. This increased monokine inducibility may contribute to the tubal damage that is the hallmark of salpingitis. (AM J OBSTET GYNECOL 1991;165:719-23.)

Key words: Salpingitis, tumor necrosis factor, monocytes, interleukin-1, interleukin-6, *Chlamydia trachomatis*

Acute salpingitis is a major cause of occlusion of the fallopian tubes and the subsequent occurrence of infertility and ectopic pregnancy. The microorganisms responsible for this disease, primarily *Neisseria gonorrhoeae* and *Chlamydia trachomatis,* and possibly a variety of other aerobic and anaerobic bacteria, have been identified.[1,2] However, the mechanism of tubal damage, especially in nongonococcal pelvic inflammatory disease, remains unclear. In organ cultures *C. trachomatis* does not visibly damage the fallopian tubes.[3] However, extensive tubal damage often may be observed in vivo in chlamydial pelvic inflammatory disease.[4]

The immune response to infection might be responsible for the tubal damage in chlamydial pelvic inflammatory disease. A single inoculation of *C. trachomatis* into the fallopian tubes of monkeys induced a transient limited infection. Repetitive inoculations, however, resulted in permanent tubal damage.[5] A 57 kd chlamydial protein, which cross reacts with heat shock proteins present in other bacterial species, has been shown to induce a potent delayed hypersensitivity response in animals previously exposed to *Chlamydia* sp.[6] Antibodies to this protein were detected in women with tubal infertility[2] and correlated with the occurrence of ectopic pregnancy in women with pelvic inflammatory disease.[7] Interferon gamma, a potent activator of cell-mediated immunity, also has been identified in the circulation of women with pelvic inflammatory disease.[8]

Products of activated monocytes and tissue macrophages, including tumor necrosis factor, interleukin-1, and interleukin-6, are potent mediators of inflammation and fibrosis.[9] Tumor necrosis factor and interleukin-1 promote infiltration by granulocytes, monocytes, and neutrophils to inflammatory sites. Interleukin-1 induces fibroblast growth and the synthesis of prostaglandin E_2 and collagenase. Tumor necrosis factor stimulates the production of toxic oxygen radicals, augments phagocytosis, interferes with epithelial cell growth, and induces interleukin-6 synthesis. Interleukin-6 induces acute phase protein synthesis and differentiation of monocytes and T and B lymphocytes.

Circulating unactivated (resting) monocytes from healthy individuals release little or no tumor necrosis factor, interleukin-1, or interleukin-6. However, if the monocytes are first exposed to interferon gamma, a product of activated T lymphocytes, or to microorganisms, they undergo a maturational change and become primed to produce monokines on subsequent exposure to low concentrations of lipopolysaccharide or bacteria that do not normally evoke a response in resting monocytes.[10]

We now report that peripheral blood mononuclear

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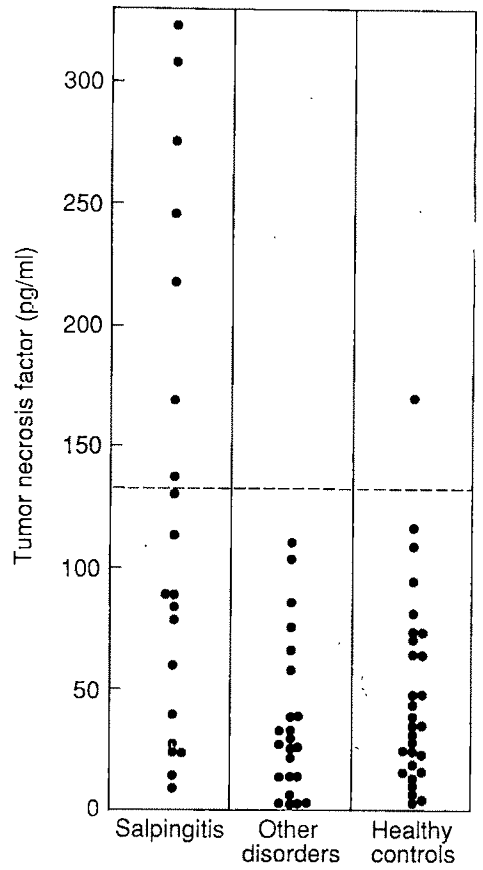

Fig. 1. Spontaneous in vitro production of tumor necrosis factor. Peripheral blood mononuclear cells from women with salpingitis or other disorders or from healthy controls were incubated for 20 hours at 37° C. Culture supernatants were collected and assayed for tumor necrosis factor by enzyme-linked immunosorbent assay. *Broken horizontal line,* Value 2 SDs above mean for healthy controls.

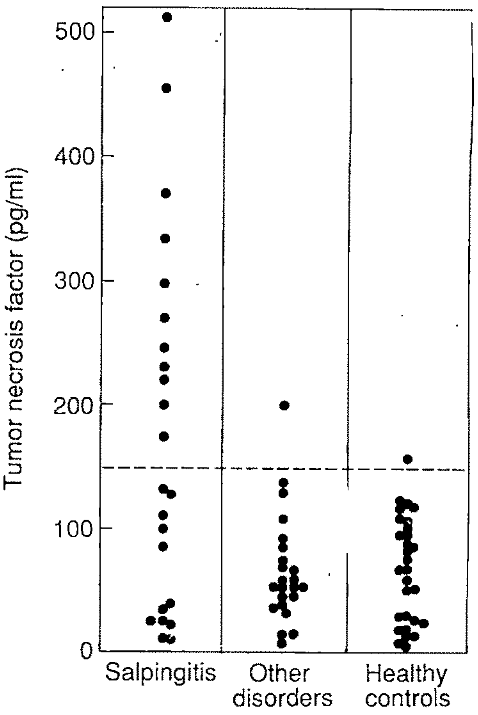

Fig. 2. Interferon gamma–induced production of tumor necrosis factor. Periperhal blood mononuclear cells from women with salpingitis or other disorders or from healthy controls were incubated in the presence of 200 U/ml of interferon gamma. Afer 20 hours, culture supernatants were collected and assayed for tumor necrosis factor. *Broken horizontal line,* Value 2 SDs above mean for healthy controls.

cells from women with salpingitis produce tumor necrosis factor-α, interleukin-1, and interleukin-6 under conditions where cells from women without salpingitis remain normally unresponsive. The results suggest that monocytes from patients with salpingitis may be primed in vivo to readily produce inflammatory mediators that may contribute to subsequent fallopian tube damage.

Material and methods

Subjects. The study population consisted of 20 patients with a current diagnosis of salpingitis and 23 other patients diagnosed with other gynecologic problems, including pelvic pain ($n = 4$), recurrent miscarriage ($n = 4$), unexplained infertility ($n = 4$), urinary tract infection ($n = 3$), recurrent vaginitis ($n = 2$), menorrhagia ($n = 2$), ovarian cyst ($n = 2$), and postpartum endometritis ($n = 2$). Controls were 29 reproductive-age women in apparently good health with no history of pelvic inflammatory disease or infection with *C. trachomatis* or *N. gonorrhoeae*. Salpingitis was diagnosed by laparoscopic surgery in 18 of the women who had visual evidence of tubal damage caused by an inflammatory process. Microbial cultures were not obtained during surgery. A differential diagnosis of salpingitis was made in two women who did not undergo laparoscopy, on the basis of the presence of adnexal tenderness, cervical motion tenderness, and identification of *C. trachomatis* in endocervical cultures. The four women with pelvic pain had no evidence of salpingitis during laparoscopy. The other patients did not undergo surgery.

Peripheral blood mononuclear cell incubation. Mononuclear cells were isolated from heparinized peripheral blood by Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) gradient centrifugation and added to multiple wells of a microtiter plate (2×10^5 cells per well) containing 10% heat-inactivated autologous serum in RPMI 1640 medium with 25 mmol HEPES buffer, L-glutamine, penicillin (100 U/ml), streptomycin (100 pg/ml), and kanamycin (100 U/ml). Interferon gamma (Genzyme, Boston) was added to triplicate wells at a concentration of 200 units/ml. After incubation for 20

Table I. Production of interleukin-1 and interleukin-6 by unstimulated peripheral blood mononuclear cells.

	Salpingitis		*Other disorders*		*Controls*	
Subject	*Interleukin-1 (pg/ml)*	*Interleukin-6 (ng/ml)*	*Interleukin-1 (pg/ml)*	*Interleukin-6 (ng/ml)*	*Interleukin-1 (pg/ml)*	*Interleukin-6 (ng/ml)*
1	149	1.8	115	1.1	73	1.9
2	174	3.0	76	1.2	110	1.6
3	73	1.0	78	1.0	167	2.7
4	136	2.0	101	1.8	109	1.8
5	169	2.7	74	1.6	73	1.6
6	140	2.0	89	1.0	109	1.6
7	134	3.0	122	2.4	89	1.0
8	104	1.7	82	1.1	74	1.5
9	379	6.3	85	1.2	80	1.3
10					85	1.3
11					89	1.1
12					94	ND

Peripheral blood mononuclear cells were incubated for 20 hours. Culture supernatants were collected and assayed for interleukin-1 or interleukin-6. *Underlined values* are >2 SDs above mean values for control group. *ND,* Not determined.

hours at 37° C in a 5% carbon dioxide incubator, culture supernatants were collected, particulate components were removed by microcentrifugation, and the supernatants were stored at $-70°$ C until used.

Monokine assays. Culture supernatants were assayed for tumor necrosis factor-α (Cistron, Pine Brook, N.J.), interleukin-1 (Cistron), and interleukin-6 (Genzyme) by enzyme-linked immunosorbent assays with commercially available kits. All samples were tested in triplicate and the mean value was determined. Replicates differed by <15%. Values were converted to picograms per milliliter by reference to a standard curve obtained by the simultaneous analysis of known concentrations of purified compounds in culture medium. The lower limit of sensitivity was 20 pg/ml for tumor necrosis factor and interleukin-1 and 0.2 ng/ml for interleukin-6.

Statistics. Differences in the numbers of women in each of the subject groups with elevated tumor necrosis factor production under the experimental conditions were evaluated by the χ^2 test, with the Yates correction factor.

Results

Spontaneous tumor necrosis factor production by peripheral blood mononuclear cells. The levels of tumor necrosis factor produced by unstimulated mononuclear cells from women with salpingitis, women with other disorders, and healthy controls are shown in Fig. 1. Only low levels of tumor necrosis factor were detected in the culture fluids of unstimulated cells from healthy women. A similar range of concentrations were detected in culture supernatants of mononuclear cells from women with disorders other than salpingitis. In marked contrast, mononuclear cells from seven of the 20 women with salpingitis spontaneously released tumor necrosis factor at levels >2 SDs above the control mean value. The difference in the number of women with elevated tumor necrosis factor production in the salpingitis and control groups was significant ($p < 0.01$).

Tumor necrosis factor production by peripheral blood mononuclear cells incubated with interferon gamma. In the presence of interferon gamma, mononuclear cells from only one of 23 patients with a diagnosis of a disorder other than salpingitis and one of 29 healthy controls produced tumor necrosis factor at a level >2 SDs above the control mean value. In marked contrast, interferon gamma–stimulated cells from 11 of 20 women with salpingitis produced tumor necrosis factor at elevated levels (Fig. 2). The number of women in the salpingitis group releasing elevated levels of tumor necrosis factor under these conditions was significantly greater than the number of women in the other groups ($p < 0.01$).

Interleukin-1 and interleukin-6 production by peripheral blood mononuclear cells from women with salpingitis. Mononuclear cells from nine patients diagnosed as having salpingitis, nine patients with other diseases, and 12 healthy controls also were tested for their ability to release two other monocyte products, interleukin-1 and interleukin-6. The upper limit of normal was designated as a value 2 SDs above the mean level of each monokine produced by peripheral blood mononuclear cells from the healthy controls; the number of patients whose unstimulated cells produced elevated levels of these two monokines was evaluated (Table I). None of the nine patients without salpingitis produced an elevated level of interleukin-1 or interleukin-6. In contrast, peripheral blood mononuclear

Table II. Production of interleukin-1 and interleukin-6 by interferon–stimulated peripheral blood mononuclear cells

	Salpingitis		*Other disorders*		*Controls*	
Subject	*Interleukin-1 (pg/ml)*	*Interleukin-6 (ng/ml)*	*Interleukin-1 (pg/ml)*	*Interleukin-6 (ng/ml)*	*Interleukin-1 (pg/ml)*	*Interleukin-6 (ng/ml)*
1	139	2.1	126	1.1	115	1.9
2	163	2.2	109	2.1	12	1.7
3	106	1.0	87	1.1	165	2.2
4	1217	6.5	134	1.8	148	1.5
5	198	2.3	88	1.7	143	1.6
6	159	2.0	102	1.0	121	1.9
7	206	2.2	147	1.9	108	1.1
8	151	1.9	118	1.2	91	1.5
9	491	3.7	91	1.1	80	1.3
10					94	1.5
11					105	1.9
12					114	ND

Peripheral blood mononuclear cells were incubated for 20 hours in the presence of 200 U/ml of interferon gamma. Culture supernatants were collected and assayed for interleukin-1 or interleukin-6. *Underlined values* are >2 SDs above mean values for control group. *ND,* Not determined.

cells from three of the patients with salpingitis spontaneously produced elevated amounts of interleukin-1, and four produced elevated amounts of interleukin-6. Similar results were obtained with interferon gamma–stimulated cells (Table II). Four of the patients with salpingitis showed elevated interleukin-1 levels and three had elevated interleukin-6 production but none of the other women did so.

In contrast to the in vitro culture results, none of the sera from patients with salpingitis contained detectable tumor necrosis factor, interleukin-1, or interleukin-6.

Comment

Peripheral blood mononuclear cells from women with salpingitis readily released products of activated monocytes in vitro under conditions where the corresponding cells from women with other gynecologic disorders or from healthy women remained relatively quiescent. Under our experimental conditions, adherence of the monocytes to the culture dishes could have been a sufficient stimulus to activate the primed monocytes to release monokines.[11] In contrast, none of the patient's sera contained detectable levels of tumor necrosis factor, interleukin-1, or interleukin-6. This is consistent with reports demonstrating that tumor necrosis factor has a short half-life and is detectable in the circulation only for a short period of time at the onset of an infection.[12] Thus monokine production in vitro by unstimulated or interferon gamma–stimulated monocytes may be a more reliable indicator of infection than the presence of these compounds in serum.

Primed monocytes present in the circulation of women with salpingitis would be highly susceptible to activation in vivo at infected tubal sites, even at loci containing low concentrations of microorganisms. Primed monocytes can be activated by levels of bacteria that are insufficient to stimulate unprimed (resting) monocytes.[10] In addition, in cases of chlamydial salpingitis, nonproductive infections also may lead to the release of chlamydial antigens capable of activating monocytes.[6] Tumor necrosis factor has, in fact, been shown to induce the disordered synthesis of chlamydial antigens.[13] The resulting accumulation of monokines at specific sites would induce scarring and tissue damage. Furthermore, since tumor necrosis factor synergizes with bacterial endotoxin in the induction of tissue necrosis,[14] tumor necrosis factor production is most likely to initiate inflammatory tissue damage if a concomitant infection is also present.

C. trachomatis infections, even those causing severe tubal damage, are often accompanied by mild or no symptoms[15] and may therefore remain undetected. In addition, the standard antibiotic treatments may not always completely clear the organism from the female genital tract.[16] Therefore low-level infections, even by micrroorganisms not normally considered to be pathogenic, may become capable of inducing monokine secretion from monocytes that were primed by "silent" chlamydial tubal infections. This might especially be a problem during pregnancy. Many studies have now shown that products of activated monocytes can induce preterm pregnant complications.[17] The reported increased incidence of first-trimester spontaneous abortion,[18] preterm labor, or premature rupture of membranes[19, 20] in women with serologic or microbiologic evidence of *C. trachomatis* also may occur, at least partially, by this mechanism.

Salpingitis is certainly not the only infectious condition in which primed monocytes are present in the circulation. Monocytes from patients with pulmonary tuberculosis[21] or meningococcal meningitis[22] also were shown to be more responsive than monocytes from controls in releasing tumor necrosis factor. Acute localized infections at other body sites would be expected to produce similar findings. However, we have not observed primed monocytes in women with vaginal or urinary tract infections. This suggests that infections confined to mucosal surfaces may not prime circulating monocytes.

The presence of a chlamydial infection is often difficult to detect clinically or by culture techniques.[2, 23] The methods described in this report may therefore be of value in the evaluation of women with suspected salpingitis.

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Maternal plasma level of endothelin is increased in preeclampsia

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Endothelin is a potent vasoconstrictor that is reportedly increased in conditions characterized by endothelial damage. Maternal plasma endothelin levels were compared between 27 women with preeclampsia (23 without and 4 with the hemolysis, elevated liver enzymes, and low platelet count syndrome) and 14 women with normotensive pregnancies. The mean ± SEM plasma endothelin values were significantly higher in patients with preeclampsia uncomplicated by the hemolysis, elevated liver enzymes, and low platelet count syndrome (5.48 ± 0.30 fmol/ml vs 3.86 ± 0.28, $p < 0.001$). In addition, the preeclamptic group with the hemolysis, elevated liver enzymes, and low platelet count syndrome had significantly higher endothelin levels than those without the syndrome (8.30 ± 1.62 fmol/ml vs 5.48 ± 0.30, $p < 0.05$). There was no correlation between plasma endothelin values and either systolic or diastolic blood pressure. We conclude that plasma endothelin levels are significantly increased in women with preeclampsia and particularly in those with the hemolysis, elevated liver enzymes, and low platelet count syndrome, suggesting an association with widespread endothelial damage. (AM J OBSTET GYNECOL 1991;165:724-7.)

Key words: Preeclampsia, endothelin, endothelial damage

There is increasing evidence that the vascular endothelium plays an important role in the regulation of vessel tone and regional blood flow.[1] Endothelin, a peptide discovered by Yanagisawa et al.[2] is produced and secreted in cultured endothelial cells and has been isolated from both porcine and human endothelial cells. Endothelin has potent vasoactive properties in vitro and is tenfold more potent than angiotensin II.[3] Plasma endothelin levels have been reported to be increased in patients with severe chronic hypertension, acute and chronic renal failure, and hemolytic uremic syndrome, implying that local endothelial cell injury may be associated with enhanced endothelin release.

Preeclampsia is characterized by vasospasm, reduced blood flow to various organs, and endothelial cell damage.[4, 5] Because endothelial cell injury and vasospasm are consistent findings in preeclampsia, we hypothesized that endothelium-derived substances such as endothelin may play a central role in the pathophysiology of preeclampsia. The purpose of this study is to compare plasma endothelin levels between patients with preeclampsia and a well-matched normotensive group.

Material and methods

The patients participating in this study were recruited from among those managed at the E.H. Crump Women's Hospital and Perinatal Center, University of Tennessee, Memphis. The study was approved by the Investigational Review Board Committee of the University of Tennessee, Memphis, and all patients gave informed consent for blood collection.

Inclusion and exclusion criteria were as follows: for the normotensive group, patients were included with a gestational age of 25 to 40 weeks, and excluded if they had any obstetric or medical complication of pregnancy and if in active labor. Patients with preeclampsia were excluded if they had other obstetric or medical complications of pregnancy and if in active labor. All patients had singleton pregnancies and none had any evidence of bleeding or fetal distress on admission.

Forty-one pregnant women between 25 and 40 weeks' gestation were studied; 14 were normotensive and 27 had preeclampsia-eclampsia. Twenty-two patients had severe preeclampsia, four had mild preeclampsia, and one had eclampsia. Four of the 22 with severe preeclampsia had the hemolysis, elevated liver functions, and low platelet count (HELLP) syndrome as defined by Sibai.[6] All patients with preeclampsia had persistent elevations in blood pressure of >140/90 mm Hg and 24 of the 27 had significant proteinuria (>300 mg/24 hour urine sample). None of the patients had prior history of chronic hypertension or renal disease.

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Table I. Clinical findings: Mean ± SD

	Normotensive $n = 14$	*Preeclampsia only* $n = 23$	*HELLP syndrome* $n = 4$
Maternal age (yr)	22.5 ± 1.2	22.4 ± 0.8	23.1 ± 1.4
Gestational age (wk)	33.1 ± 1.1	33.4 ± 1.1	31.9 ± 1.3
Systolic blood pressure (mm Hg)*	118 ± 9.2	168 ± 13.7	157 ± 16.4
Diastolic blood pressure (mm Hg)*	68 ± 8.9	105 ± 10.7	95 ± 4.1
Uric acid (mg/dl)	NA	6.2 ± 0.33	7.35 ± 0.41
Platelet count ($\times 10^3/\text{mm}^3$)	NA	242 ± 16†	44 ± 7†
SGOT (IU/L)	NA	38 ± 7†	280 ± 86†

SGOT, Serum glutamate oxaloacetate transaminase.

*$p < 0.001$ By analysis of variance.

†$p < 0.001$ By the Student t test.

Twelve of the normotensive women were nulliparous, whereas 24 of the 27 women with preeclampsia were nulliparous. Two of the three multiparous women had the HELLP syndrome; the other patient had eclampsia. Blood pressure was measured in a semirecumbent position with a standard mercury sphygmomanometer with an appropriate-sized cuff that was positioned at the level of the heart. Two blood pressure recordings were obtained immediately before blood collection. The average of these two recordings was used in the regression analysis of the endothelin levels.

Blood samples were obtained during either the antepartum period or the latent phase of labor in two normotensive patients and two patients with preeclampsia. Peripheral venous blood samples for endothelin were collected into chilled tubes containing 5 mg of ethylenediaminetetraacetic acid per milliliter and 500 U of aprotinin per milliliter. The plasma fraction was removed after immediate centrifugation at 4° C and subsequently stored at −70° C until assayed. At the same time, routine laboratory tests for preeclampsia, such as uric acid level, liver function, or coagulation studies were obtained with the same venipuncture.

Plasma endothelin concentrations were measured with a radioimmunoassay technique (Amersham Corporation, Arlington Heights, Ill.) after purification and concentration of plasma samples. Briefly, 3 ml of plasma were acidified and loaded into a small chromatography column (Sep Pak, C_{18}, Millipore Corporation, Millford, Mass.). The column was washed sequentially with (4%) acetic acid and distilled water before immunoreactive endothelin was eluted with methanol. Each sample in methanol was evaporated to dryness under a stream of nitrogen and the residue dissolved in 250 μl of assay buffer that was included in the kit. Duplicate 100 ml aliquots were taken for assay. The sensitivity of the assay (2 SD of zero-point binding) was 1.03 fmol. Intraassay and interassay coefficients of variation were 4.1% and 10.4%, respectively. The antiserum recognizes both endothelin 1 and endothelin 2.

Statistical analysis was done with analysis of variance or the Student t test where appropriate. Plasma endothelin levels were compared with the Mann-Whitney U test. A p value of <0.05 was considered significant.

Results

Table I summarizes the clinical findings among the normotensive women and those with preeclampsia with and without HELLP syndrome. There were no significant differences in maternal or gestational age at the time of blood sampling between the normotensive and preeclamptic groups.

Fig. 1 describes the plasma endothelin levels among the three groups of patients. Plasma endothelin levels were significantly higher in patients with preeclampsia without the HELLP syndrome than the levels in the normotensive group (5.48 ± 0.30 fmol/ml vs 3.86 ± 0.28, $p < 0.001$). In addition, patients with HELLP syndrome had significantly higher endothelin levels than did patients with preeclampsia without the HELLP syndrome (8.30 ± 1.62 fmol/ml vs 5.48 ± 0.30, $p < 0.05$).

Among the preeclamptic group, there was no correlation between maternal plasma endothelin values and systolic blood pressure, diastolic blood pressure, platelet count, uric acid, or serum glutamate oxaloacetate transaminase levels.

Comment

Endothelin 1 is a 21-residue vasoconstrictor peptide that has been isolated from the supernatant of porcine aortic endothelial cells.[2] It is one of a family of peptides that are produced in mammals, including humans. Endothelin 1 has a wide range of regulatory functions, particularly cardiovascular, renal, respiratory, and endocrine. Endothelin 1 also has a potent mitogenic ac-

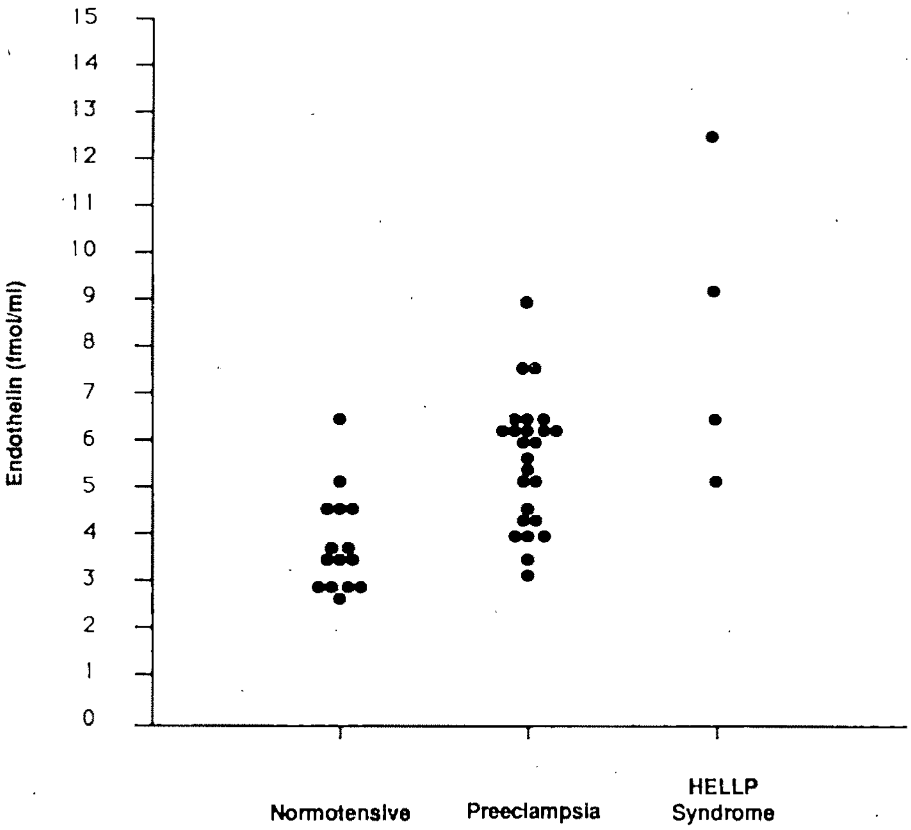

Fig. 1. Distribution of plasma endothelin levels among the three groups of patients. Preeclampsia versus normotensive ($p < 0.001$); preeclampsia versus HELLP syndrome ($p < 0.05$).

tivity on fibroblasts and smooth muscle cells.[1] The other family members, endothelin 2 and endothelin 3, have not been precisely characterized in their biologic activity. Only endothelin 1 has been recovered from endothelial cells. Endothelin 1 has a long-lasting vasopressor action, and it is tenfold more potent than angiotensin II, thus being the most potent vasoconstrictor yet identified.[3] The kidney is particularly sensitive to its vasoactive effects, which decrease both glomerular filtration rate and urine output.[7]

Increased plasma levels of endothelin 1 have been reported in several disease states characterized by endothelial damage and coagulopathy, such as hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, and acute renal failure.[8] In addition, elevated maternal plasma levels of endothelin have been reported in preeclampsia.[9, 10] Kamoy et al.[9] reported elevated plasma levels of endothelin 1 in eight women with pregnancy-induced hypertension and lower plasma endothelin 1 levels in pregnancy compared with the nonpregnant state. Taylor et al.[10] reported elevated maternal plasma endothelin 1 in 10 preeclamptic patients at term; however, in patients who had serial blood sampling during pregnancy, plasma endothelin 1 levels were not increased before the clinical development of preeclampsia.

There is evidence of vascular endothelial injury in preeclampsia, both from ultrastructural studies[5] and from biochemical studies: increased fibronectin[11] as well as factor VIII antigen.[12] In addition, the increased fibronectin develop long before the clinical onset of the disease. Thus the endothelial response to injury may antedate and contribute to the occurrence of preeclampsia.

Our findings reveal a significant increase in plasma endothelin levels in patients with preeclampsia, particularly in those with the HELLP syndrome. Endothelial cell damage is believed to be widespread in these patients, with the resulting microangiopathy, platelet consumption, and disseminated intravascular coagulopathy. The endothelin levels in preeclampsia did not correlate with either maternal systolic or diastolic blood pressure values. However, endothelin levels correlated with the presence of HELLP syndrome. These findings suggest that endothelin 1, which is increased in conditions associated with endothelial damage, may be considered a marker of endothelial damage with pathophysiologic significance in preeclampsia.

Endothelial cells are capable of releasing a variety of local mediators such as prostacyclin, endothelium-derived relaxing factor, tissue plasminogen activator, fibronectin, heparan sulfate, platelet-activing factor, and endothelin 1.[1] Some of these mediators appear to exert a counterregulatory effect on each other. For example, the pressor effects of circulating endothelin are limited by the release of prostacyclin and endothelium-derived relaxing factor,[13] indicating that the potent and long-lasting pressor effect of endothelin is modulated by the endothelial release of potent vasodilating and antiaggregatory agents.

Under conditions of endothelial damage, such as preeclampsia, the increased endothelin 1 levels may lead to progressive vasospasm with eventual reduction in both renal plasma flow and uteroplacental blood flow. In addition, the increased endothelin 1 levels may be responsible for the increase in mitogenic activity reported in preeclampsia.[14]

In summary, endothelin is increased in patients with preeclampsia, particularly in those with HELLP syndrome. Further research on endothelin 1 and other related endothelial mediators such as prostacyclin and endothelium-derived relaxing factor may enable us to better understand the endothelial response to circulating injury in preeclampsia.

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In utero fetal muscle biopsy for the diagnosis of Duchenne muscular dystrophy

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Deoxyribonucleic acid techniques can be used to diagnose Duchenne muscular dystrophy prenatally in male fetuses that are at risk. Deoxyribonucleic acid–based prenatal diagnosis can be impossible when there is only one prior affected male and there is no identifiable deletion or alteration. We performed fetal muscle biopsy in utero in such a case and documented the presence of dystrophin, thereby confirming normality in a male fetus at risk. This first in utero experience adds fetal muscle biopsy to the available procedures for fetal tissue diagnosis. (AM J OBSTET GYNECOL 1991;165:728-32.)

Key words: Duchenne muscular dystrophy, prenatal diagnosis, in utero muscle biopsy

Duchenne muscular dystrophy is a progressive, degenerative muscle disease that is inherited as an X-linked recessive trait. Affected boys have an unremarkble early childhood, with the first overt clinical symptoms appearing around 2 to 5 years of age. Most patients are confined to a wheelchair by the age of 10 and usually die by the age of 20. It is one of the more common genetic diseases among all populations of the world. Affecting approximately one in 3500 live-born male infants, Duchenne muscular dystrophy is caused by the deficiency of a minor component of muscle tissue, called dystrophin.[1-3] Dystrophin helps form the membrane cytoskeleton in normal muscle, and its deficiency results in myofiber necrosis, presumably because of membrane instability.

Approximately 70% of cases are inherited through a carrier mother, whereas 30% of cases show no previous family history and therefore represent de novo mutations of the dystrophin gene.[4] This high spontaneous mutation rate of the dystrophin gene may result from the enormous size of the gene, which is nearly an order of magnitude larger than all other genes studied to date.[5] This high occurrence rate of spontaneous mutations poses problems in genetic counseling. In those women with no prior family history and normal creatine kinase levels, a Bayesian probability of 30% for having future subsequent sons affected with Duchenne muscular dystrophy can be calculated.

The recent isolation of the dystrophin gene[6] and identification of its normal protein product[1] have prompted the advent of what has been called molecular diagnostics for this disorder.[7] Specifically, the actual gene mutation causing Duchenne muscular dystrophy can be identified in 65% of affected individuals as a gene deletion.[1] In these families female relatives carrying the same mutation can be identified. Prenatal diagnosis can then be offered by analyzing the deoxyribonucleic acid (DNA) isolated from chorionic villi or amniotic fluid cells for the presence or absence of the same deletion mutation that was identified in the affected family member.

However, in the remaining 35% of families who do not have deletion mutations, both carrier detection and prenatal diagnosis are more difficult and less accurate. In such cases, restriction fragment length polymorphism analyses must be undertaken in an attempt to mark the affected X chromosome in the family. If only a single affected family member exists, it is often impossible to determine whether the mother of the affected boy is a carrier. This uncertainty has ramifications not only for future pregnancies of the mother but also for the carrier risks of other at-risk female relatives. With Bayesian analysis only, the probability of being a carrier can be generated. If the male fetus at risk of being affected with Duchenne muscular dystrophy inherits an X chromosome from his mother that is different from the previously affected male, then, with the exception of the small possibility of crossing over in meiosis, the diagnosis of Duchenne muscular dystrophy can be excluded.[8] However, if the male fetus inherits the *same* X chromosome as his previously affected sib, the risk that the fetus is affected is the same

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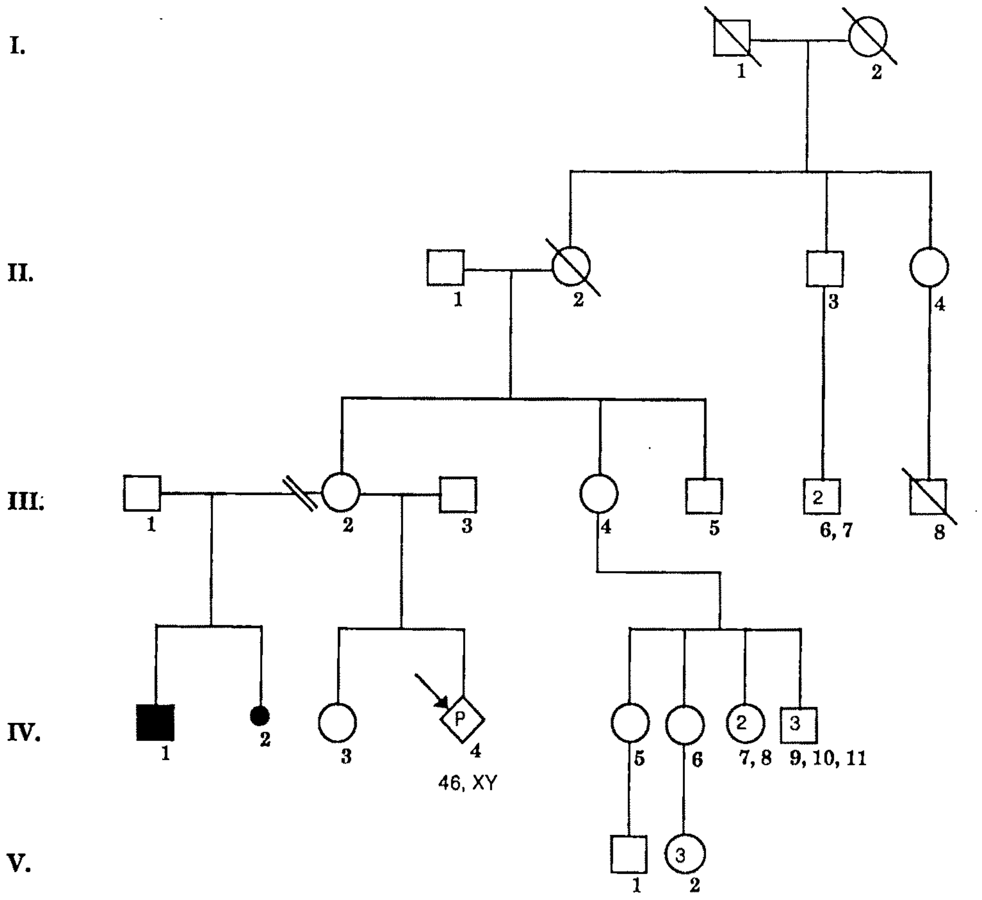

Fig. 1. Pedigree of family. III 2 is proband who had a 19-year-old son (IV 1) with Duchenne muscular dystrophy. ■, Affected male subject.

as the risk that the mother is, in fact, a carrier. Such uncertainty in risk assessment has been seen in other X-linked disorders, such as hemophilia.[9]

We describe such a genetically *difficult* family, where an unborn male fetus has inherited the same X chromosome as his brother who has Duchenne muscular dystrophy, has no dystrophin gene deletion, and apparently represents a sporadic case. Thus the risk that the fetus will have Duchenne muscular dystrophy is the same as the risk that the mother will be a carrier, which is approximately 30%. To avoid termination of this high-risk pregnancy, a novel method, namely, dystrophin protein testing of the fetal muscle in utero,[8] was performed and was diagnostic. As this was an innovative approach, this procedure was cleared with the department chairman and research committee, but a formal university institutional review board approval was not necessary for a single therapeutic attempt, given that fetal tissue biopsies are long-standing, accepted procedures. The procedure was not attempted on a patient seeking termination, for example, because that would violate federal regulations of pregnancy and require institutional review board approval. The operator (M.I.E.) felt competent on the basis of thousands of invasive procedures performed, as to the technical feasibility of the procedure. This first case of successful in utero muscle biopsy and the molecular diagnostic methods used are described in this report.

Case report

A 41-year-old white woman, gravida 4, para 2, spontaneous abortion 1 (Fig. 1, III 2), was referred to the Division of Reproductive Genetics at Hutzel Hospital–Wayne State University for genetic counseling and chorionic villus sampling because of advanced maternal age. However, analysis of the family history first revealed a 19-year-old son (IV1) with Duchenne muscular dystrophy with follow-up for many years by a Duchenne muscular dystrophy clinic. Further analysis of the family history revealed that he was the only family member affected with the disease. The proband also has an unaffected daughter. Her sister had two unaffected sons. The proband had creatine kinase levels determined on three occasions in the last 5 years, all of which fell within the normal range. At her initial visit at 9 weeks' gestation, she underwent chorionic villus sampling. Karyotype revealed a 46,XY chromosomally normal male. Blood was obtained for molec-

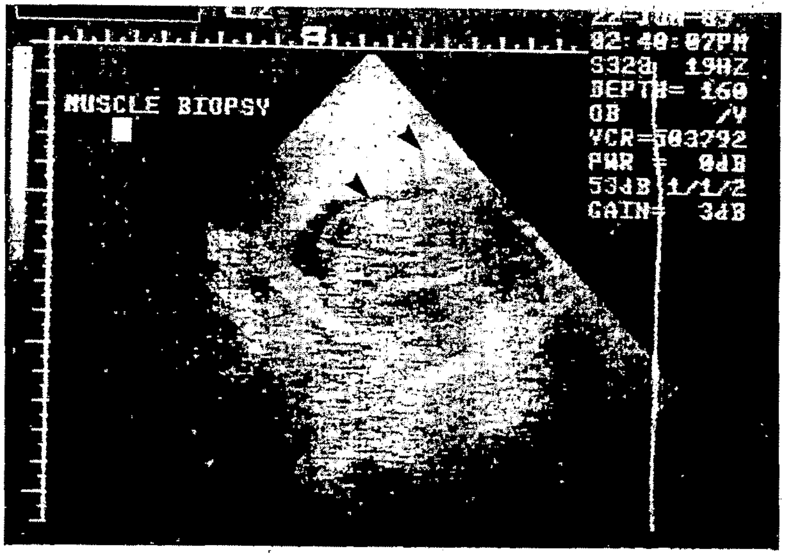

Fig. 2. Sonogram showing biopsy forceps through skin over buttock as biopsy was taken. *Left arrow,* Tip of forceps inside buttock. *Right arrow,* Shaft outside of fetus.

Table I. DNA analysis

Enzyme × *probe*	*IV 1*	*III 2*	*Male fetus*
Pst I × 754	+	+ +	
Eco RV × pERT 87-1	+	[+ −]	+
Bgl II × complementary DNA 30-1	−	− −	
Bgl II × complementary DNA 47-4	+	+ +	
Msp I × P20	−	− −	
Eco RV × C7	+	[+ −]	+
Haplotype designation	A	AB	A

ular diagnosis and analyzed at The Johns Hopkins Hospital.

Although 65% of males with Duchenne muscular dystrophy have a dystrophin gene rearrangement, usually with a partial deletion causing them dystrophy, none of the major reported rearrangements were seen in the DNA of IV 1. Thus it was not possible by this type of analysis to determine whether the mother was a carrier and the fetus was at risk.

Restriction fragment length polymorphism haplotype analysis was undertaken to determine whether the male fetus of III 2 had inherited her at-risk haplotype. Analysis was informative at two restriction fragment length polymorphisms, an *Eco*RV site that was detectable with probe C-7 that flanks the gene on the 3′ side and an internal *Eco*RV site that was detected by the intragenic probe pERT 87-1. IV 1 and the fetus (IV 4) had inherited the same allele type at both of these markers (Table I). Thus the chance that the fetus was affected with Duchenne muscular dystrophy was approximately the same that III 2 was, in fact, a carrier. By Bayesian calculations, taking into account the family pedigree and the three normal creatine kinase results obtained on III 2, the chance that she was a carrier was calculated to be approximately 30%. Thus the chance that her fetus was affected with Duchenne muscular dystrophy also was approximately 30%.

The option of pregnancy termination followed by dystrophin analysis[1-3] and possible reattempted pregnancy was offered to the patient. However, she hoped for more information and did not want to risk aborting a normal male fetus. The option of attempting a fetal muscle biopsy was presented, and the patient gave informed consent for the innovative approach. The consent included our uncertainty of the likelihood of being successful and the unknown risks of the procedure.

Under ultrasonographic guidance at approximately 17 weeks 5 days' gestation, a Klear Kut (Baxter, Los Angeles) kidney forceps gun was inserted through a nick incision in the skin and into the uterine cavity. The choice of Klear Kut instrument was one of convenience because it is used routinely in our hospital. The Klear Kut device is useful in having a shearing, but specimen-protecting, feature necessary to assure that the specimen is not lost when the needle is removed from the uterus. Several other similar devices are available and have been used for similar procedure. The forceps was positioned for entry into the gluteal muscle in a downward fashion over the buttock (Fig. 2). The forceps was advanced inside the skin, and a core biopsy of muscle was obtained. The buttocks were thought to be easier to use than the calf because they are larger and less likely to move. By sampling on the outside and posteriorly, the risk of nerve injury can be reduced but certainly never eliminated. The specimen was then sent to Boston Childrens Hospital for dystrophin analysis. Unfortunately, the specimen was thought to be inadequate because of insufficient muscle tissue content as determined by Coomassie Brilliant Blue G staining of myosin heavy chain protein. The procedure was re-

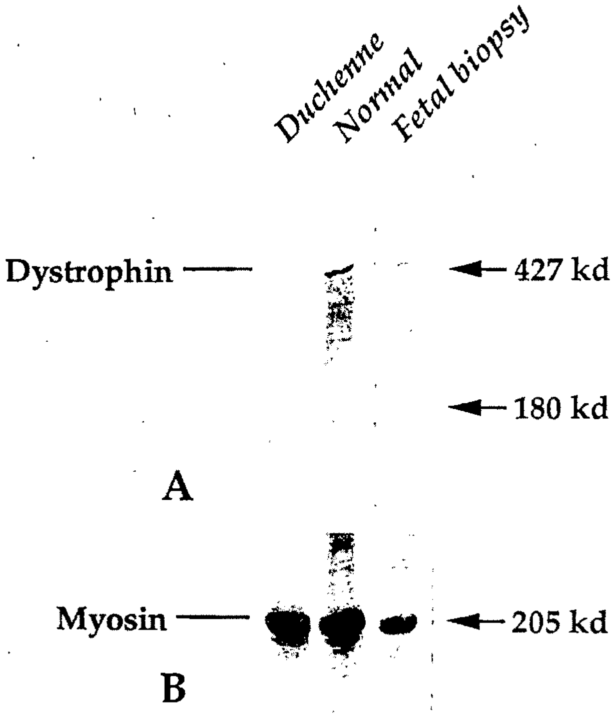

Fig. 3. Dystrophin immunoblot analysis of fetal biopsy. Shown is dystrophin analysis *(panel A)* and myosin heavy chain analysis *(panel B)* done sequentially on the same immunoblot. *Duchenne* lane contains total muscle protein from 5-year-old patient with Duchenne muscular dystrophy who has deletion encompassing his entire dystrophin gene. *Normal* lane contains muscle from normal adult. *Fetal biopsy* lane contains total solubilized protein from proband discussed in this report.

peated again at 19 weeks without difficulty; an adequate muscle specimen was obtained, and analysis of the biopsy for dystrophin content found a normal amount of the dystrophin protein, compared with control levels, indicating that the fetus was not affected with Duchenne muscular dystrophy (Fig. 3). Ironically, the 19-year-old son (IV 1) died of respiratory failure the next week after the diagnosis was made.

The remainder of the pregnancy was uneventful. At 40 weeks she was delivered of a 7 pound 15 ounce male infant with Apgar scores of 3 at 1 minute and 7 at 5 minutes. He was observed initially in the neonatal intensive care unit because of apneic spells, but he went home within 1 week with follow-up on an apnea monitor and has done well ever since. Examination of the buttocks revealed a small scar from the puncture site. Creatine kinase levels were within normal limits (44 IU/L) at 6 months of age.

Comment

Genetic analyses, by either deletion or linkage studies, are informative and accurate in the large majority of families. However, specific cases often arise whereby genetic analyses prove uninformative or inconclusive. Such situations often occur when the affected family member is not available for dystrophin gene analysis, or, as in the case reported here, if there is a sporadic case with no detectable gene deletion. In a previous report dystrophin testing of an at-risk abortus was successfully used to determine whether the proband was a carrier and to identify the Duchenne muscular dystrophy–carrying X chromosome.[8] Dystrophin testing of at-risk abortuses has been subsequently used successfully in many cases where DNA analysis has proved ambiguous (Hoffman EP. Unpublished observations).

Clearly, it is not an ideal situation if a pregnancy must be terminated to determine the maternal carrier status or whether the fetus is affected, or both. In the case presented here termination of an at-risk pregnancy was at least postponed in favor of in utero muscle biopsy. The muscle biopsy was successful and demonstrated that the fetus was, in fact, normal, even though it had inherited the same X chromosome as an affected sibling. Thus we were able to show that the affected individual in this family was indeed a sporadic case and that the mother was not a carrier of Duchenne muscular dystrophy, except for the small chance of gonadal mosaicism.

The development of invasive biopsy techniques for fetal tissues first began in the early 1980s, when fetoscopic blood sampling, skin biopsies, and liver biopsies were first attempted.[10] With the development of very sophisticated ultrasonographic technology, visualization of the fetus has superceded previous fiberoptic scope techniques. Thus most invasive procedures are now done with ultrasonographic guidance. Furthermore, the development of molecular techniques has obviated the need for tissue products in many cases, such as in ornithine transcarbamylase deficiency, which previously required a fetal liver biopsy.[11] Fetal tissue sampling for diagnosis will still be important, because in many disorders molecular approaches may not be available or measurement of gene products may be necessary to adequately diagnose or gauge the effectiveness of therapy. Whether 17 weeks is too early to have a reasonable chance of a successful biopsy is unclear. Further experience will be needed to evaluate the efficacy of the procedure and the optimum timing. Fortunately, only tiny quantities of muscle are needed for the laboratory analysis.

From this first successful experience, it is impossible to gauge accurately the risks of an in utero fetal muscle biopsy. It clearly would be preferable to do procedure-related DNA diagnoses of Duchenne muscular dystrophy from chorionic villus sampling or amniocentesis, because of both earlier gestational ages at which the procedures can be performed and their demonstrated safety. However, the development of the ability to do fetal muscle biopsies adds a second dimension beyond sex selection and Bayesian calculations to the procedures for diagnosis of such cases when DNA diagnosis is inconclusive or otherwise impractical.

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Concentration of digoxin-like immunoreactive substance in patients with preeclampsia and its relation to severity of pregnancy-induced hypertension

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Digoxin-like immunoreactive substance (endoxin) was measured in pregnant patients with pregnancy-induced hypertension. The mean level was significantly higher than that in healthy control subjects ($p < 0.001$). Patients with eclampsia had a significantly higher concentration than did women with preeclampsia ($p < 0.03$). A positive significant correlation was found between concentration of digoxin-like immunoreactive substance and the value of the index of gestosis of von Goecke and Schwabe ($p < 0.03$). Analysis of patterns during various kinds of therapy revealed that nitrendipine was most effective in lowering endoxin concentration. Although an insignificant correlation was found between maternal concentration and both birth and placental weights, the compartmental differences observed seem to suggest fetoplacental origin of this substance. (Am J Obstet Gynecol 1991;165:733-6.)

Key words: Digoxin-like immunoreactive substance, endoxin, pregnancy-induced hypertension, antihypertensive treatment

Preeclampsia complicates 5% to 7% of all pregnancies and is associated with increased risk for both mother and newborn. The precise cause of pregnancy-induced hypertension remains obscure; a variety of cofactors are being considered.[1-7] During the last decade much attention has been paid to natriuretic substances such as atrial natriuretic factor and digoxin-like immunoreactive substance, and their role in water and sodium retention has been studied.[8-11]

The aim of this study was to test the relationship between the digoxin-like immunoreactive substance serum concentration in patients with preeclampsia and the severity of the pregnancy-induced hypertension syndrome. Additionally, the influence of various modes of pharmacologic therapy on serum digoxin-like immunoreactive substance patterns was investigated.

Material and methods

The 85 patients enrolled in the study were divided into five groups (Table I). The control group ($n = 45$) consisted of nonpregnant healthy subjects and pregnant women with uncomplicated pregnancies. The study group consisted of 40 patients with pregnancy-induced hypertension in the third trimester. Patients with chronic renal disease, essential hypertension, or liver disfunction and those receiving digitalis glycosides were excluded from the study. Diagnosis of preeclampsia was based on the criteria established by the Committee on Terminology of the American College of Obstetricians and Gynecologists.[12] The severity of pregnancy-induced hypertension was calculated according to the index of gestosis of von Goecke and Schwabe[13] (Table II). Gestational age was determined by means of the last menstrual period and physical examination and was confirmed by ultrasonographic biometry.

Patients with preeclampsia were randomly divided into three groups according to the mode of treatment: 11 patients receiving nitrendipine, 20 mg four times daily, 23 patients receiving dihydralazine, 25 mg six times daily and six patients treated with continuous intravenous infusion of 50 mg Largactil, 50 mg diphenhydramine hydrochloride, and 100 mg of meperidine hydrochloride in 500 ml of 5% dextrose. Three determinations of maternal concentration of digoxin-like immunoreactive substance were made: after 24 hours of bed rest and before medication, after 3 days of therapy, and immediately after delivery. The interval between the second and third estimation varied from 5 to 14 days. In 15 cases digoxin-like immunoreactive substance concentrations were estimated in the umbilical cord vein and in the newborn serum within 24 hours post partum and correlated with birth and placental weights (Table III). Digoxin-like immunoreactive substance concentrations were estimated after serum deproteinization with a mixture of 5-sulfosalicilic acid and 50% methanol in equal parts by means of the fluorescence polarization immunoassay method with

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Table I. Clinical data for healthy nonpregnant and pregnant women and preeclamptic patients

		Healthy women				
	Nonpregnant women (n = 10)	*Pregnant, first trimester (n = 10)*	*Pregnant, second trimester (n = 10)*	*Pregnant, third trimester (n = 15)*	*Preeclamptic patients, third trimester (n = 40)*	*p Value*
Age (yr)	25.0 ± 0.6	23.8 ± 0.8	24.1 ± 0.5	23.1 ± 0.8	23.6 ± 0.7	NS
Gestational age (wk)	—	9.9 ± 1.2	18.8 ± 0.8	39.2 ± 1.3	36.7 ± 4.4	<0.001*
Blood pressure (mm Hg)						
Systolic	119.0 ± 2.1	118.5 ± 2.5	112.3 ± 4.6	123.1 ± 4.5	167.1 ± 16.9	<0.001*
Diastolic	71.3 ± 1.8	68.2 ± 2.0	65.7 ± 5.2	73.2 ± 5.8	104.6 ± 6.7	<0.001*
Digoxin-like immunoreactive substance	0.0	0.0	0.0	0.22 ± 0.2	0.57 ± 0.23	<0.001*

All values are given as mean ± SD.

*Statistically different when healthy pregnant women in third trimester are compared with preeclamptic patients.

Table II. Scheme of index of gestosis of Goecke and Schwabe

	Score			
Symptoms	*0*	*1*	*2*	*3*
Arterial blood pressure (mm Hg)				
Systolic	≤140	>140-160	>160-180	>180
Diastolic	≤90	>90-100	>100-110	>110
Proteinuria (gm/L, Esbach's method)	<1	1-3	>3-6	>6
Edema	Absent	Weak on lower legs	Strong on lower legs	Generalized

Severity of preeclampsia and summary of scores: light, 1-5 middle, 6-9 severe, 10-12.

Table III. Mean newborn and placenta weights and digoxin-like immunoreactive substance concentrations in various compartments

				Digoxin-like immunoreactive substance (ng/ml)	
	Birth weight (gm)	*Placental weight (gm)*	*Umbilical cord blood*	*Newborn venous blood*	*Maternal venous blood*
Healthy women in third trimester (*n* = 9)	3498.8 ± 333.4	652.0 ± 99.0	0.73 ± 0.14	0.51 ± 0.21	0.37 ± 0.1
Preeclamptic patients (*n* = 15)	3300.0 ± 512.8	653.5 ± 150.6	1.00 ± 0.31*	0.82 ± 0.35*, †	0.43 ± 0.17

All values are given as mean ± SD.

*Statistically different from healthy control ($p < 0.02$).

†Umbilical cord blood versus newborn venous blood; correlation coefficient, 0.55; $p < 0.03$.

the Abbott TDx system II (Abbott Diagn. Inc., North Chicago). The lower limit of assay sensitivity was 0.2 ng/ml. Concentrations of digoxin-like immunoreactive substance below this value were considered immeasurable. The statistical analysis was performed with the Student t test after homogeneity of variances was ascertained and by means of the Pearson correlation coefficient. A p value <0.05 was considered statistically significant.

Results

Digoxin-like immunoreactive substance concentrations in healthy nonpregnant women and healthy pregnant women in the first and second trimesters were immeasurable. Pregnant women with an uncomplicated course of gestation in the third trimester had mean concentrations that were more than twice as low as those found in patients with preeclampsia in the same stage of pregnancy (Table I). Furthermore, when we divided patients with pregnancy-induced hypertension into two subgroups, preeclamptic and eclamptic, we found that in the latter group the digoxin-like immunoreactive substance concentration was much higher (0.83 ± 0.29 ng/ml) than that seen in the first subgroup (0.56 ± 0.21 ng/ml), and this difference was statistically significant ($p < 0.03$) (Fig. 1). The severity

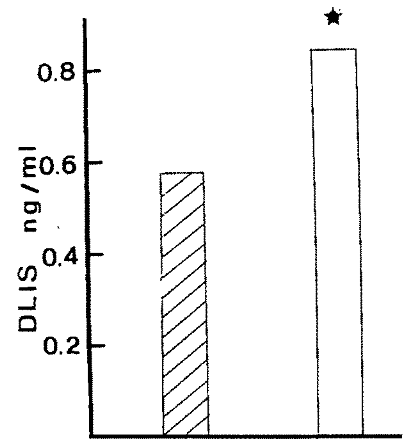

Fig. 1. Digoxin-like immunoreactive substance concentration in preeclamptic and eclamptic patients. *Vertical bar,* Preeclamptic patients; *open bar,* eclamptic patients; *asterisk,* statistically different from preeclamptic patients ($p < 0.03$).

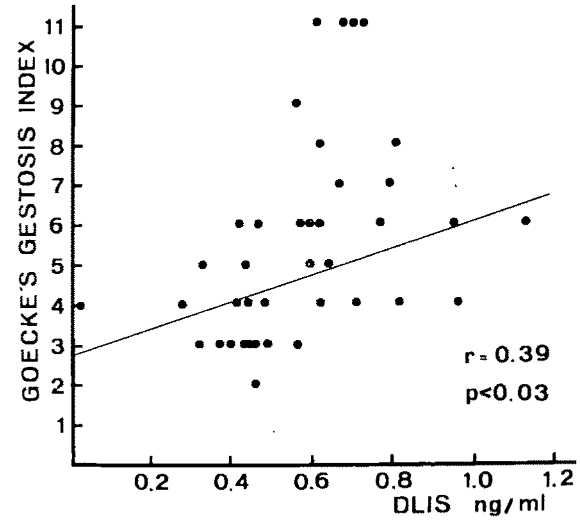

Fig. 2. Correlation between severity of pregnancy-induced hypertension measured as index of gestosis of von Goecke and Schwabe and maternal digoxin-like immunoreactive substance concentration.

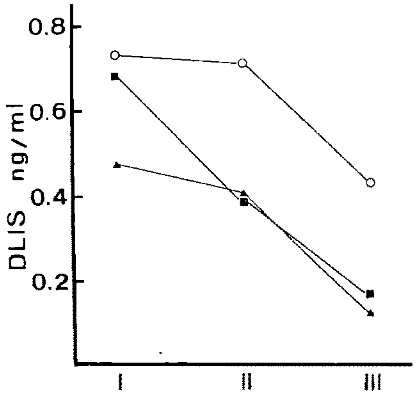

Fig. 3. Digoxin-like immunoreactive substance patterns during various kind of therapy. *Open circles,* Patients treated with Largactil + diphenhydramine hydrochloride + meperidine hydrochloride; *squares,* patients treated with nitrendipine; *triangles,* patients treated with dihydralazine. *I, II, III,* intervals between follow-up of digoxin-like immunoreactive substance estimations.

of pregnancy-induced hypertensive syndrome in our preeclampsic patients, measured as von Goecke and Schwabe's index of gestosis, was correlated with the serum digoxin-like immunoreactive substance concentration (Fig. 2). The statistical analysis of serum digoxin-like immunoreactive substance concentrations seen during the course of various kinds of therapy revealed that nitrendipine was most effective in lowering levels of the substance in the group of preeclamptic patients (Fig. 3). In the preeclamptic patients a positive correlation was found between digoxin-like immunoreactive substance concentration in cord blood serum and newborn venous blood in the first day of life, whereas such a correlation was not present in the group of healthy women. In both investigated groups we were unable to show any correlations between serum digoxin-like immunoreactive substance concentrations and newborn or placenta weights (Table III).

Comment

Digoxin-like immunoreactive substance was found in serum, urine, amniotic fluid, and placental homogenate with advancing pregnancy.[10, 11, 15] Gusdon et al.[16] and Valdes[10] have found significantly higher concentrations of digoxin-like immunoreactive substance in the serum of preeclamptic women, whereas Gonzalez et al.[17] and Phelps et al.[18] did not confirm these observations. Probably the discrepancies found in previously published data might be explained at least partially by the various analytic methods used, as shown by Pudek et al.[19] In our material we confirmed observations of the previously mentioned authors[10, 16] that digoxin-like immunoreactive substance concentration was significantly higher in groups of preeclamptic patients. Moreover, eclamptic patients had even higher levels than were found in preeclamptic women. The positive significant correlations between digoxin-like immunoreactive substance concentration and severity of the pregnancy-induced hypertensive syndrome, measured as the index of gestosis of von Goecke, that we found might suggest that this substance(s) is somehow involved in the pathogenesis of preeclampsia, most probably by the influence on membrane sodium-potassium-adenosine triphosphatase activity. To our knowledge this is the first report showing such a correlation in patients with pregnancy-induced hypertension. Goodlin[11] reported no detectable endoxin level in cord blood samples of anencephalic newborns and showed that bethamethasone therapy decreased the concentration of this substance; this might support the fetal adrenal origin of endoxin. In every case in our material endoxin concentration after delivery was markedly decreased in the manner reported previously by Gusdon et al.[16] confirming fetal origin of this particular substance(s). Endoxin has been proposed as an inhibitor of sodium, potassium, and calcium exchange and therefore as a peripheral vasoconstrictor agent.[20] Although several treatment regimens have been proposed for pregnancy-induced hypertension syndrome, no consideration has been given to maternal endoxin concentration. When we related modes of pharmacologic treatment to digoxin-like immunoreactive substance concentration pattern during therapy, we found that the calcium channel blocker nitrendipine was not only effective as a hypotensive agent but also the most effective in lowering the serum concentration of digoxin-like immunoreactive substance in preeclamptic patients. Because we found correlation between levels of digoxin-like immunoreactive substance in the cord and venous blood of newborns of preeclamptic patients and because such a correlation was not present when maternal digoxin-like immunoreactive substance concentrations were taken into consideration, we assumed that this factor (or factors) was of fetal origin. A special question is that concerning biologic activity of digoxin-like immunoreactive substance in humans during pregnancy complicated by pregnancy-induced hypertension and how we can influence sodium-potassium-adenosine triphosphatase membrane transport by direct manipulation of endoxin concentration. In one published case[21] a patient with eclampsia was treated with a specific antidigoxin antibody (Digibind), and reduction of digoxin-like immunoreactive substance concentration accompanied by antihypertensive effect was obtained.

Our results have shown that the severity of pregnancy-induced hypertension syndrome positively correlates with the maternal serum concentration of digoxin-like immunoreactive substance. Therefore the level of this substance might be of importance as an indicator of antihypertensive treatment effectiveness in preeclamptic patients.

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A new proline aminopeptidase assay for diagnosis of bacterial vaginosis

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Bacterial vaginosis is one of the most common occurring vaginal conditions among women of reproductive age. A rapid and reliable laboratory test for diagnosis of bacterial vaginosis would be helpful in the clinical detection of this disease. Elevated proline aminopeptidase activity has been identified as a reliable marker enzyme for bacterial vaginosis. A proline aminopeptidase assay has been shown to predict accurately women with a clinical diagnosis of bacterial vaginosis. However, this assay has significant practical disadvantages, the most notable of which is the production of a carcinogenic end product, α-naphthylamine. We have developed a modified assay for this bacterial vaginosis marker enzyme with L-proline *p*-nitroanilide, a substrate that does not yield a carcinogenic end-product. The new proline aminopeptidase assay is a one-step test that is analyzed colorimetrically with microsomal leucine aminopeptidase used as a standard enzyme (linear from 3 to 125 mU per well). We have determined the activity of proline aminopeptidase in vaginal wet preparations from 57 patients with both assay methods. In addition, vaginal smears were examined with Gram's stain and analyzed for bacterial vaginosis with the Spiegel method. When compared with the Spiegel method, the two proline aminopeptidase assay methods were similar with respect to assay sensitivity (93%), specificity (91% to 93%), and the predictive value of a positive result (78% to 82%) or a negative result (97% to 98%). Vaginal wash samples also were assessed for proline aminopeptidase activity. Values for samples identified as bacterial vaginosis positive were significantly different ($p < 0.0001$) from those that were negative according to the Spiegel analysis of Gram's stain: negative results, 66 ± 41 mU/ml; positive results, 704 ± 145 mU/ml. These findings indicate that this improved proline aminopeptidase assay will offer a rapid, sensitive, and objective laboratory method for the diagnosis of bacterial vaginosis. (AM J OBSTET GYNECOL 1991;165:737-42.)

Key words: Bacterial vaginosis, nonspecific vaginitis, microbiology of the vagina, anaerobic bacteria

Bacterial vaginosis is one of the most common vaginal conditions in reproductive age women.[1] It accounts for approximately one third of all cases of vaginitis in women of reproductive age and is one of the most frequent complaints of women who visit their primary care physician. Bacterial vaginosis has been implicated in several gynecologic diseases and disorders, including recurrent urinary tract infections,[3] adnexal tenderness,[4] postpartum endometritis,[5] and increased risk of infection after gynecologic surgery.[6] In addition, the consistent and persistent association of bacterial vaginosis with placental infection, intraamniotic infection, preterm labor, premature rupture of membranes, and low birth weight[7-11] underscores the importance of developing a suitable diagnostic tool for the early detection and treatment of this vaginal condition. Devising a rapid and sensitive diagnostic test to confirm a diagnosis of bacterial vaginosis would facilitate objective and reliable detection of this condition. Such a test would also enhance the ability to study both putative complications of bacterial vaginosis and new treatment initiatives for this condition. The ideal test for bacterial vaginosis would have only a few simple steps, require no special equipment, have good assay sensitivity, specificity, and predictive value, and would be available commercially at a reasonable price.

Bacterial vaginosis is characterized by several changes in the normal microecologic and physicochemical environments of the vagina. This shift in vaginal flora from a predominance of *Lactobacillus* morphotypes to an overgrowth of anaerobic bacteria yields the production of bacterial metabolites that are unique to the vaginal fluid of women with bacterial vaginosis.[12] These factors have provided the basis for several methods of diagnosis of bacterial vaginosis. Clinical diagnosis of bacterial vaginosis, which is based on an evaluation of vaginal fluid and pH, has been difficult to standardize.

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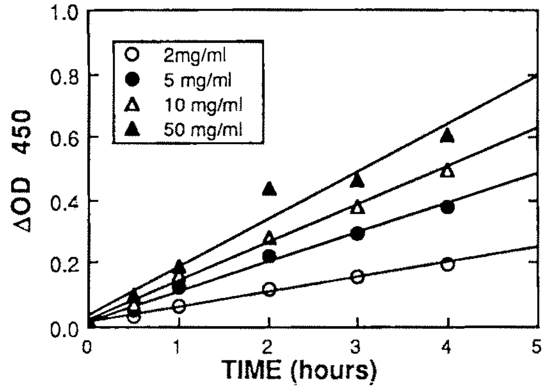

Fig. 1. Effect of increasing concentration of L-proline *p*-nitroanilide in proline aminopeptidase assay. Substrate concentration was increased from 2 to 5, 10, or 50 mg/ml. Reaction was initiated by addition of 62.5 mU of leucine aminopeptidase to each well. Assay was incubated at 37° C. Sample absorbances were read at 450 nm at 0.5, 1, 2, 3, and 4 hours.

The clinical parameters evaluated are somewhat subjective and vary with the adequacy of the specimen and the skill of the practitioner. The clinical diagnosis also may be ambiguous, as a result of multiple infections, menstruation, large amounts of cervical mucus, recent douching, or sexual intercourse before a pelvic examination.

Four laboratory methods also have been used to diagnose bacterial vaginosis: (1) culture of vaginal fluid for *Gardnerella vaginalis*,[13] (2) gas-liquid chromatographic analysis of vaginal fluid fatty acids[14, 15] and amines[16, 17] that are believed to be products of anaerobic bacterial growth, (3) Gram's stained vaginal smears that are read microscopically for bacterial morphotypes,[18, 19] and (4) assay for proline aminopeptidase (Enzyme Commission 3.4.11), a marker enzyme that is present in vaginal secretions and reliably elevated in bacterial vaginosis.[20]

In this study we sought to improve the proline aminopeptidase assay by eliminating some of the practical disadvantages inherent in the original assay design.[20] The substrate L-proline β-naphthylamide, when cleaved by proline aminopeptidase, yields the highly carcinogenic end product β-naphthylamine[21]; the assay reaction requires the secondary addition of a dye to visualize this end product. Finally, as published,[20] this test is purely qualitative; no standard reaction curves with β-naphthylamide were available for quantitation of the end product. The aims of this study were to develop a quantitative proline aminopeptidase assay with a substrate that does not yield a carcinogenic end product and to compare this new proline aminopeptidase assay with that of Thomason et al.[20] and with the standardized Gram's stain method of Spiegel et al.[19]

Material and methods

Samples were obtained from women enrolled in a larger study of lower genital tract microorganisms and prematurity.[22] A detailed history and physical examination were obtained from consenting women who were between 26 and 30 weeks' gestation. During speculum examination, samples of vaginal fluid were obtained from the midvaginal lateral wall with sterile cotton swabs. One swab was rolled onto a glass slide, and the sample was allowed to air dry for Gram's stain and evaluation for the presence of bacterial vaginosis. A second cotton swab saturated with vaginal fluid was placed in 1 ml of sterile Dulbecco's buffered saline solution. This vaginal "wet prep" was evaluated microscopically for motile trichomonads and yeast pseudohyphae, then frozen at −70° C for analysis with the proline aminopeptidase assay. Finally, the posterior vaginal fornix was irrigated with 4 ml of sterile Dulbecco's buffered saline solution. After standardized mixing, saline solution containing vaginal fluid was aspirated into a sterile syringe. These vaginal wash specimens were held initially at 4° C and frozen at −70° C within 4 hours of collection.

Vaginal smears were evaluated for bacterial vaginosis by a single investigator (S.L.H.), without knowledge of other findings, using the criteria established by Spiegel et al.[19] Vaginal smears were determined to be consistent with the presence of bacterial vaginosis on the basis of two criteria: (1) five or fewer *Lactobacillus* morphotypes per oil field and (2) more than five *G. vaginalis* morphotypes plus one other bacterial morphotype (gram-positive cocci, *Bacteroides* morphotypes, or curved rods). Analyses were scored as either positive or negative for bacterial vaginosis.

All chemicals used in the proline aminopeptidase assay were obtained from Sigma Chemical Company (St. Louis). When they had thawed, vaginal "wet prep" samples were centrifuged at 1000 *g* for 15 minutes to obtain a cell pellet. All but 100 μl of the supernatant was decanted, and the cell pellet was resuspended in the remaining supernatant. This suspension was then transferred to a microtiter plate well containing 50 μl of a 2 mg/ml solution in pH 7.0 Tris buffer of either L-proline β-naphthylamide (Thomason substrate) or L-proline *p*-nitroanilide (new substrate). After a 4-hour incubation at 37° C, fast garnet GBC dye was added to each well in the Thomason assay. After a second incubation for 5 minutes at room temperature, this assay was read visually.[20] A salmon red–pink color indicated a diagnosis of bacterial vaginosis. An orange-yellow color was scored negative for bacterial vaginosis. In the new proline aminopeptidase assay, samples were read visually immediately after the 4-hour incubation. A yellow color indicated a diagnosis of bacterial vaginosis; a

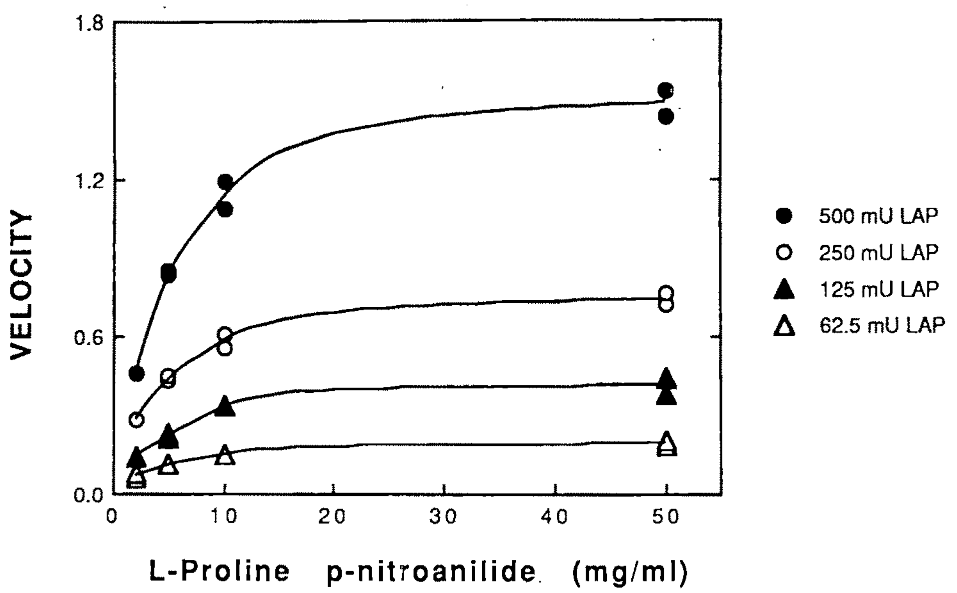

Fig. 2. Reaction velocity at 30 minutes as a function of substrate concentration. Increasing amounts of the substrate L-proline *p*-nitroanilide (2, 5, 10, and 50 mg/ml) were incubated with leucine aminopeptidase (62.5 to 500 mU per well). Sample absorbances (450 nm) were read after a 30-minute incubation at 37° C.

clear color was scored as negative for bacterial vaginosis.

Vaginal wash samples obtained from these patients also were analyzed for proline aminopeptidase activity. Before processing, 0.6 ml of each vaginal wash sample was centrifuged to obtain a cell pellet, and fluid in excess of 0.3 ml was discarded. The remaining 0.3 ml of fluid and cells were resuspended, and 0.1 ml aliquots were pipetted into microtiter plate wells; this allowed the determination of proline aminopeptidase activity in triplicate for each sample. Proline aminopeptidase activity was assessed quantitatively in these vaginal wash samples according to the new assay protocol. Use of L-proline *p*-nitroanilide as the chromogenic substrate allowed spectrophotometric quantitation of the assay end products. The presence of the yellow end product, nitroanilide, was measured spectrophotometrically at 450 nm. Because a standard preparation of proline aminopeptidase is not available, a standard curve was constructed for quantitative analysis with the use of microsomal leucine aminopeptidase (Enzyme Commission 3.4.11.1). This enzyme is specific for the amino terminal of peptides and will cleave proline from the substrate L-proline *p*-nitroanilide in a linear fashion from 3 to 125 mU/well. One unit of leucine aminopeptidase activity was presumed to be equal to 1 U of proline aminopeptidase. Under the standard conditions described, the assay limit of detection (defined as twice the assay background) was 0.02 U of absorption at 450 nm.

Assay sensitivity and specificity were calculated for each assay, with the Spiegel Gram's stain score used as the "gold standard" point of reference. Student's *t* test was used for statistical comparison of vaginal wash sample values.

Results

The kinetics of the enzyme-substrate reaction are shown in Figs. 1 and 2. The appearance of end product over time was examined for substrate concentrations ranging from 2 to 50 mg/ml (Fig. 2). The yellow-colored end product, nitroanilide, increased linearly (as evidenced by the increase of sample optical density at 450 nm) as a function of time, indicating that the reaction was run under conditions of substrate excess during the reaction time examined. Increased substrate concentration (up to 10 mg/ml) was beneficial in terms of the assay sensitivity; a deeper yellow color was apparent earlier in the incubation. A substrate concentration of 50 mg/ml yielded a high background that diminished assay sensitivity. Reaction velocity at 30 minutes is plotted as a function of substrate concentration in Fig. 2. At a substrate concentration of ≥10 mg/ml, the reaction velocity was at its maximum.

All clinical data described here were generated under the standard assay conditions of 2 mg/ml substrate incubated with the vaginal sample for 4 hours at 37° C. Table I shows the comparison of Gram's stain analysis and the two proline aminopeptidase assays that were run under standard conditions. A total of 57 vaginal samples were examined. Gram's stain analysis indicated that 42 samples were negative for bacterial vaginosis

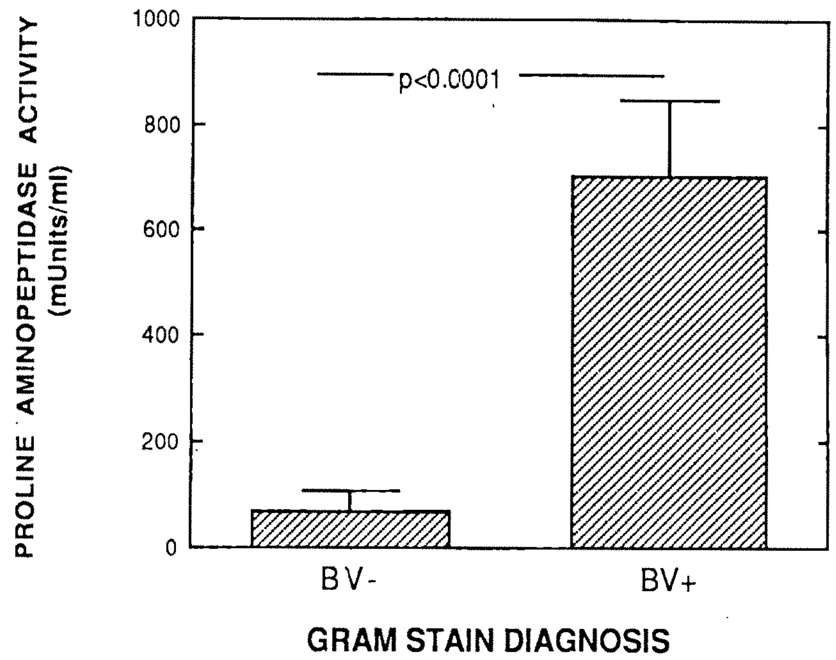

Fig. 3. Proline aminopeptidase activity (mean ± SE, milliunits per milliliter) in vaginal wash samples. Values from 57 vaginal samples are grouped according to Spiegel Gram's stain analysis.

Table I. Comparison of diagnosis: Gram's stain versus proline aminopeptidase assays

Method	*Diagnosis of bacterial vaginosis*	
	Positive	*Negative*
Gram's stain	42	15
Proline aminopeptidase assay		
L-Proline β-naphthylamide	39	18
L-Proline *p*-nitroanilide	40	17

and 15 were positive. The diagnoses that were based on the two proline aminopeptidase assays were remarkably similar, 39 and 40 were negative for bacterial vaginosis and 17 and 18 were positive for bacterial vaginosis. When compared with the Gram's stain results, proline aminopeptidase assay sensitivity was 93% and specificity was 91% to 93%.

Proline aminopeptidase activity was significantly elevated in vaginal wash samples obtained from women with bacterial vaginosis as determined by Gram's stain (Fig. 3; $p < 0.0001$, unpaired t test). The activity (mean ± SE) associated with bacterial vaginosis was 704 ± 145 mU/ml ($n = 15$). Absence of bacterial vaginosis was associated with a very low level of proline aminopeptidase activity (mean ± SE, 66 ± 41 mU/ml, $n = 42$).

Comment

These data demonstrate that the two proline aminopeptidase assay substrates examined, L-proline β-naphthylamide and L-proline *p*-nitroanilide, yield similar results with respect to a diagnosis of bacterial vaginosis. When compared with the results of Gram's stain, both proline aminopeptidase assays yielded similar findings. The proline aminopeptidase assay sensitivity (93%) was well within the range of acceptable values for a reliable diagnostic test. Assay specificity was also acceptable at 91% to 93%. The revised proline aminopeptidase assay offers noncarcinogenic substrate, L-proline *p*-nitroanilide.

This new proline aminopeptidase assay also has the capacity for quantitation of vaginal proline aminopeptidase activity. Our data show that activity of this enzyme was significantly elevated in women with bacterial vaginosis, as determined by Gram's stain analysis. There was, however, some variability apparent in proline aminopeptidase activity present in vaginal wash samples that may be attributed to the variability inherent in this sampling method. Vaginal fluids are variably diluted, depending on the amount of fluid present before introduction of saline solution into vagina. Also, the recovery of saline solution and fluid removed from the vagina is difficult to standardize.

In the report of Thomason et al.,[20] proline aminopeptidase in vaginal fluid correlated well with a positive clinical diagnosis of bacterial vaginosis. In our hands there is also an excellent correlation between diagnosis of bacterial vaginosis by Gram's stain and the presence of proline aminopeptidase, whether the assays employed proline β-naphthylamide or L-proline *p*-nitroanilide. Although it has not yet been carefully investigated, proline aminopeptidase is presumed to be a product of the bacteria associated with bacterial vaginosis. Mobiluncus and *G. vaginalis* have been shown to produce proline aminopeptidase (Hillier SL. Unpublished data). Other genera known to produce proline aminopeptidase include *Actinomyces*, *Streptococcus* (viridans streptococci), and *Peptostreptococcus*.[23] Additional work is needed to identify the specific organism(s) responsible for the synthesis of this enzyme in the setting of bacterial vaginosis. In addition, the vaginal conditions that are specific to bacterial vaginosis and are conducive to production and secretion of this marker enzyme remain to be determined.

Several important advantages of the modified assay for proline aminopeptidase serve to recommend it as an outstanding candidate for further use and validation in the clinical setting. Use of L-proline *p*-nitroanilide provides a simple one-step assay; vaginal fluid can be placed directly into a well containing the substrate. This assay is relatively rapid; it can be completed within 4 hours. The assay is objective; a positive or negative diagnosis can be determined visually or, if the equipment is available, spectrophotometrically.

Clinical diagnosis of bacterial vaginosis is currently widely used; it includes an assessment of the appearance of the vaginal fluid, determination of pH, detection of an amine odor, and microscopic examination of a wet mount of vaginal fluid. Unfortunately, the necessary equipment and expertise are not always available to clinicians. Thus access to simple laboratory methods for the diagnosis of bacterial vaginosis would be valuable.

Several laboratory analyses have been used to detect metabolites associated with bacterial vaginosis and to confirm a clinical diagnosis of bacterial vaginosis.[15-17, 20] These methods, however, require expensive equipment and reagents not easily available to clinicians.

Microscopic examination of Gram's stained vaginal smears has proved practical and economical. Diagnosis of bacterial vaginosis by Gram's stain criteria has been shown to be superior to diagnosis that is based on vaginal cultures in both pregnant and nonpregnant women.[12, 14, 19, 24] However, the subjectivity and dependence on investigator skill that are inherent to the Gram's stain analysis[24] suggest the need for a more objective method for the diagnosis of bacterial vaginosis.

The isolation of *G. vaginalis* also has been examined as a predictor of bacterial vaginosis. *G. vaginalis* can be recovered from 83% to 94% of women with clinical signs of bacterial vaginosis.[12] The presence of *G. vaginalis*, however, is not restricted to women with bacterial vaginosis.[1, 4, 12, 14] Accordingly, while the isolation of *G. vaginalis* has high sensitivity, it has low specificity and low predictive value of a positive culture. Krohn et al.[12] reported the consistent association of bacterial vaginosis with increased isolation of *G. vaginalis, Bacteroides* spp., and *Mycoplasma hominis* and with decreased isolation of facultative lactobacilli. Although this microbial pattern appears to be a good predictor of bacterial vaginosis, the microbiologic methods for culture and identification of these bacteria are time-consuming and costly and require a degree of expertise that is not universally available.

In spite of the currently available methods for diagnosis of bacterial vaginosis, there clearly remains a need for a quick, easy, and objective method to confirm a clinical diagnosis of bacterial vaginosis. The optimum means of screening for the presence of bacterial vaginosis remains to be determined. Both proline aminopeptidase assays offer rapid, easy, and objective means for the diagnosis of bacterial vaginosis. Although the number of samples analyzed in this study is limited, our findings suggest that further clinical validation of this new proline aminopeptidase assay is worthwhile.

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Pemphigus gestationis (herpes gestationis): A case report

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Pemphigus gestationis is a rare disease, probably of immunologic origin, that causes the placenta to absorb immunoglobulins and deposit them in its basement membrane. The resulting placental insufficiency leads to increased morbidity and mortality among the offspring of such patients. A case of pemphigus gestationis is presented, and the importance of early diagnosis is discussed. (AM J OBSTET GYNECOL 1991;165:743-4.)

Key words: Pemphigus gestationis

Pemphigus gestationis is a rare disease unrelated to any known viral infection. Its incidence has been roughly estimated to be 1 in 10,000 deliveries. The disease is probably of immunologic origin and consists of pruritic, polymorphic, inflammatory, subepidermal bullous dermatosis of pregnancy and the puerperium. The following is a report of a case of pemphigus gestationis, its workup, and its treatment during pregnancy.

Case report

A 26-year-old, gravida 2, para 1 woman was admitted to the dermatologic ward at 28 weeks' gestation. Her medical history and course of pregnancy until admission had been uneventful. At 27 weeks' gestation she began to notice what she described as mosquito bites located around the umbilicus and on the right foot.

On admission she had a highly pruritic papulovesicular eruption that involved the abdomen, legs, and soles. Later the hands and face were involved, as well. The lesions varied from erythematous, edematous papules to large bullae with different intermediate forms (Fig. 1). A biopsy of the perilesional area was performed and pemphigus gestationis was diagnosed by means of histopathologic examination and direct immunofluorescence.

Treatment with 20 mg per day of cortisone taken by mouth was immediately initiated, without any apparent response. When dosage was increased to 60 mg per day of cortisone, she responded well. Special attention was given to the fetal growth pattern, which was measured by serial ultrasonographic examination. Flow examination results were within the normal range, biweekly nonstress test results were reactive, and growth pattern was normal.

At 35 weeks' gestation the patient had regular uterine contractions and was delivered of a live male infant weighing 2850 gm with Apgar scores of 10 at 1 minute and 10 at 5 minutes. The weight of the placenta was 580 gm. A biopsy performed on a sample taken from the placenta did not reveal deposits of the third component of complement (C3) in the basement membrane.

The patient was rehospitalized immediately post partum because of a severe exacerbation and again was successfully treated with cortisone and antihistamines. Human leukocyte (HLA) antigen typing was performed, and the patient was found to have a phenotype of A1, A30, B35, B5, Cw4, DR3.

The infant had a similar eruption that subsided within weeks without special treatment.

Comment

Although rare, the two most common types of bullous dermatosis in pregnancy are pruritic urticarial papules and plaques of pregnancy and pemphigus gestationis.

The exact cause of pemphigus gestationis is unknown, but the consistent finding of C3 deposits at the basement membrane zone of lesional and normal-appearing skin, along with the findings of an avid component-fixing immunoglobulin G antibody in the serum, implies a role for immunologic mechanisms. A genetic predisposition has also been proposed for patients in the HLA-B8, DR3, and DR4 phenotypes.

When physicians are confronted with bullous dermatosis, it is essential to differentiate pemphigus gestationis from pruritic urticarial papules and plaques of pregnancy. In contrast to pruritic urticarial papules and plaques of pregnancy, which does not seem to recur in subsequent pregnancies and does not increase fetal morbidity, there is a great increase in the number of small newborns and in infant mortality among the offspring of mothers with pemphigus gestationis.[1]

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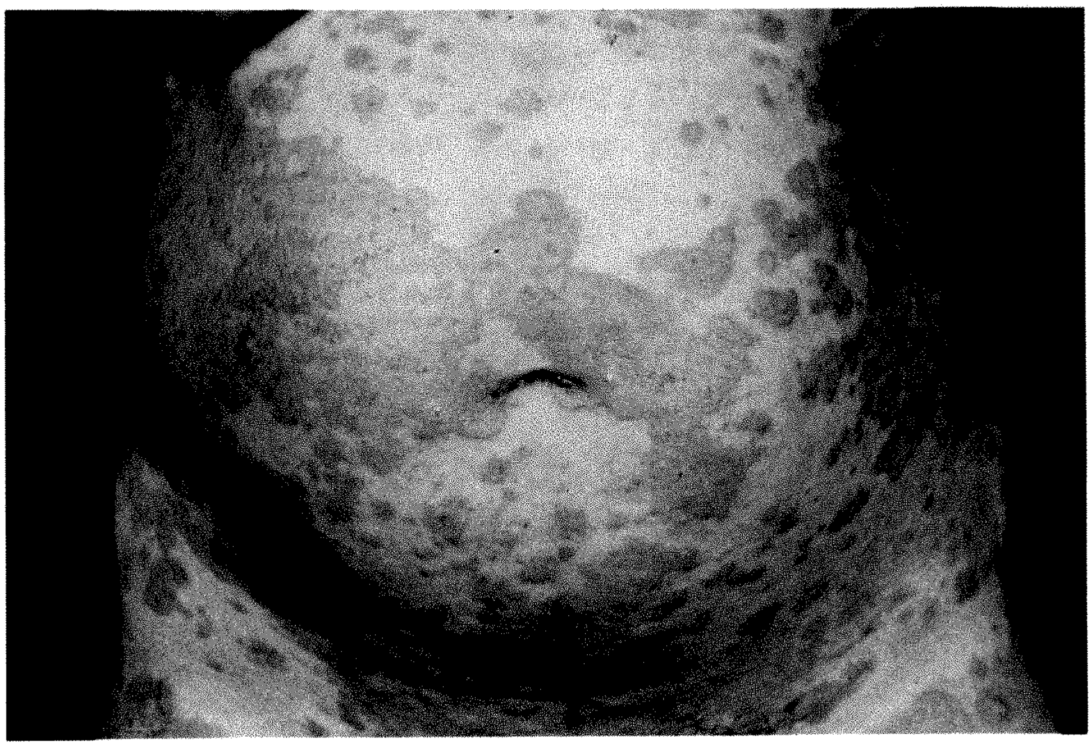

Fig. 1. Papulovesicular eruption on abdomen and flanks of woman with pemphigus gestationis at 28 weeks' gestation.

Direct immunofluorescence studies of the peribullous and urticarial lesions or of normal-appearing skin demonstrate heavy homogeneous liner deposition of C3 along the basement membrane zone with concomitant immunoglobulin G deposits in 30% to 40% of patients.

Indirect immunofluorescence assay showed the sera of patients with pemphigus gestationis to contain an antibody that avidly binds complement to the basement membrane of normal skin (pemphigus gestationis factor).

The fetus remains relatively protected from the immune complex formation because the placenta absorbs the immunoglobulin and deposits it within the placental basement membrane.[2] This phenomenon results in an increased frequency of placental insufficiency, which causes increased morbidity and mortality in the offspring of patients with pemphigus gestationis.

The treatment of pemphigus gestationis consists of systemic corticosteroid administration, which is usually quite effective in relieving the pruritus and in suppressing new lesion formation.

There is a high frequency of postpartum exacerbation, and physicians must be prepared to quickly reinstitute or increase therapy at the time of delivery.

Fewer than 5% of the infants of mothers with pemphigus gestationis will have vesiculobullous lesions, which are usually mild and transient. Infants exposed to high doses of corticosteroids should be monitored for adrenal insufficiency.

The immediate and vigorous treatment in our case probably inhibited the formation and deposition of the immune complexes in the placenta and therefore prevented placental insufficiency, although the maternal disease was fulminant.

A pregnancy complicated by pemphigus gestationis should be considered to be a high-risk pregnancy and should be monitored and managed accordingly.

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Pelvic denervation for chronic pain associated with endometriosis: Fact or fancy?

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The efficacy of presacral neurectomy and amputation of the uterosacral ligaments in the treatment of chronic pelvic pain has been debated for decades. These procedures used to be done mainly in women with normal pelves, but more recently they have been performed during conservative surgery for treatment of endometriosis. In the 1980s the rapid spread of laparoscopic surgery has led to an increasing number of endoscopic denervations in patients with chronic pelvic pain associated with endometriosis. However, an analysis of literature data has failed to prove that presacral neurectomy and amputation of the uterosacral ligaments are effective and did not demonstrate better results with the use of lasers rather than electrocoagulation. Moreover, no valid comparison has yet been made between laparotomy and laparoscopic methods. (AM J OBSTET GYNECOL 1991;165:745-9.)

Key words: Pelvic pain, dysmenorrhea, dyspareunia, endometriosis, presacral neurectomy

Chronic pelvic pain is frequently associated with endometriosis.[1-3] Most studies on conservative treatment of this disease have considered the results mainly in terms of fertility, and the most effective management of pain symptoms in women wanting children is still a matter of debate.

In 1899 the Italian Ruggi[4] and the Frenchman Jaboulay[5] published the first descriptions of presacral nerve resection in women with severe dysmenorrhea. During the first half of this century presacral neurectomy was performed increasingly in both Europe[6] and the United States.[7-9] In the 1950s Doyle[10] demonstrated the efficacy of paracervical denervation for the relief of dysmenorrhea by abdominal or vaginal transection of uterosacral ligaments. Until then, pain in women with normal pelves had rarely been differentiated from pain associated with pathologic conditions such as chronic pelvic inflammatory disease or endometriosis. The advent of nonsteroidal antiinflammatory drugs allowed effective treatment of most women with "essential" dysmenorrhea. Consequently, during the 1960s and 1970s there was a fall in the number of pelvic denervations, which were performed mainly during conservative surgery for severe pelvic endometriosis. During the 1980s it gradually became clear that the possibilities of medical "cure" for endometriosis were limited[11, 12] and interest in pelvic denervation underwent a corresponding resurgence, enhanced by the progressive widespread use of operative endoscopy performed with high-tech methods.[13]

Different views have alternated with various generations of surgeons, but the basic problem is still unresolved: Does the performance of pelvic denervation in addition to the standard excision of ovarian and peritoneal lesions significantly increase the well-being of patients with endometriosis or only increase the risk of intraoperative and postoperative morbidity?

We have analyzed the recent literature data in an attempt to clarify the usefulness of presacral neurectomy and amputation of the uterosacral ligaments in women with chronic pelvic pain associated with endometriosis, comparing the pain relief obtained by classic and endoscopic surgery.

Pelvic denervation at laparotomy

Few clinical studies have been designed to evaluate the efficacy of conservative laparotomy for endometriosis specifically in terms of pain relief (Table I). Furthermore, the disease stage has not always been specified.

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Table I. Pelvic denervation for chronic pain associated with endometriosis: Effects on symptoms according to literature

		Dysmenorrhea		*Dyspareunia*		
Series	*Treatment*	*No. with symptoms*	*% Experiencing relief*	*No. with symptoms*	*% Experiencing relief*	*Type of study*
Garcia and David,[14] 1977	CSEL + PSN	35	97	26	74	Retrospective, nonrandomized
	CSEL	36	72	34	39	
Puolakka et al.[15] 1980	CSEL + PSN	51	90	NR	—	Retrospective, nonrandomized
	CSEL	45	80	NR	—	
Polan and DeCherney,[16]* 1980	CSEL + PSN	20	70	NR	—	Retrospective, nonrandomized
	CSEL	19	26	NR	—	
Lee et al.[17]* 1986	CSEL + PSN	19	79	15	87	Retrospective, nonrandomized
	CSEL + PSN + USLR	21	67	19	68	
Tjaden et al.[18] 1990	CSEL + PSN	17	88	11	55	Prospective, partly randomized
	CSEL	9	0	5	60	
Feste,[19] 1985	LPS + carbon dioxide laser + USLR	32	72	NR	—	Observational
Davis,[20] 1986	LPS + carbon dioxide laser + USLR	146	92	109	94	Observational
Daniell,[21] 1989	LPS + KTP laser + USLR	80	75	NR	—	Observational
Sutton and Hill,[22] 1990	LPS + carbon dioxide laser + USLR	187	70	NR	—	Observational
Perez,[23] 1990	LPS + carbon dioxide and Nd:YAG laser + USLR	25	80	NR	—	Observational

CSEL, Conservative surgery for endometriosis at laparotomy; *PSN,* presacral neurectomy; *NR,* not reported; *USLR,* uterosacral ligament resection; *LPS,* laparoscopy; *KTP,* potassium-titanium-phosphorus; *Nd:YAG,* neodymium:yttrium-aluminum-garnet.

*Mixed pelvic disease.

Garcia and David[14] performed a retrospective analysis of postoperative pain symptoms in 71 patients who underwent conservative surgery for endometriosis and found that in the 35 women who underwent presacral neurectomy dysmenorrhea and dyspareunia decreased significantly with respect to the 36 controls (97% vs 72% and 74% vs 58%). In another retrospective study Puolakka et al.[15] compared 45 women undergoing simple resection of endometriotic lesions with 51 in whom presacral neurectomy also was performed. Pain symptoms resolved or were markedly attenuated in 80% of the former and 90% of the latter. Although the authors considered a 10% difference to be of importance, it does not seem statistically significant.

In Polan and DeCherney's study[16] the combination of presacral neurectomy with conservative surgery in women with chronic pelvic pain, endometriosis, or pelvic inflammatory disease increased the percentage of total postoperative pain relief from 26% to 75%, but the mixed pelvic disorders and the small number of patients studied make it difficult to draw conclusions. Lee et al.[17] performed presacral neurectomy in 50 women with chronic pelvic pain, and dysmenorrhea resolved in 73% of the cases, dyspareunia lessened in 77%, and acyclic pain showed improvement in 63%. The uterosacral ligaments were resected in half of the subjects in the study, but this did not improve the pain relief rates. Again no definitive conclusions can be based on this study because of the inclusion of mixed pelvic disorders.

Recently, Tjaden et al.[18] published the combined results of a randomized study and a comparative nonrandomized study performed in patients with moderate or severe endometriosis and chronic pelvic pain who underwent conservative surgery with or without presacral neurectomy. These authors divided dysmenorrhea into midline and lateral categories on the basis of the differences in sensory innervation and observed that midline pain was abolished in all four patients randomized to neurectomy versus none of the four controls who underwent only resection of pelvic endometriotic lesions. These data, together with similar findings in a group of 18 nonrandomized women, led the monitoring committee to stop the study because "it was considered unethical to continue to deprive patients with midline dysmenorrhea of the benefit of pain relief that could be afforded with presacral neurectomy." Somewhat surprisingly, there was no difference between cases and controls in relief of dyspareunia.

Because of numerous biases in the design of most of the studies, constructive comments cannot be made on laparotomy for treatment of pelvic pain in patients with endometriosis. Almost all the reports are retrospective and observational without a randomized control group, the extent of endometriosis is rarely indicated, sometime different pelvic diseases are accumulated (such as endometriosis and pelvic inflammatory disease), the sample sizes are limited, the follow-ups are variable, and an objective evaluation of the pain symptoms on the basis of a multidimensional verbal score and/or a visual analogue pain scale[24, 25] has almost never been performed prospectively. Recently, amputation of the uterosacral ligaments was considered in a report[17] in which the authors observed similar results in women who underwent neurectomy regardless of whether the uterosacral ligaments were or were not resected. Thus whether similar pain relief is achieved by resection of the uterosacral ligaments alone and by presacral neurectomy is still an open question. This could be interesting since in women with endometriosis the uterosacral ligaments may constitute a specific source of disabling pain.[26, 27] Furthermore, resection of the uterosacral ligaments at laparotomy is simple and safe, once the ureters are identified.[28, 29] Presacral neurectomy may result in morbidity, including severe bleeding,[17, 30] and constipation.[31] In the absence of verified advantages this operation could be limited to patients in whom identification of the uterosacral ligaments is difficult because of extensive endometriosis of the pouch of Douglas. Tjaden et al.[18] concluded that when the results of neurectomy are unsatisfactory, resection was incomplete or selection of the patients was inappropriate. On this last point Slocumb[32] has recently written that ". . . the success of presacral neurectomy reported in the literature is not consistent with the general clinical impression of ob/gyn practitioner and teaching faculty . . . in chronic pelvic pain patients, the localization of pain in terms of tender tissues rarely is limited to the uterosacrals and uterus alone. Many of these other areas not only are outside the areas of presacral innervation, but also are more amenable to other methods of pain treatment."

Pelvic denervation at laparoscopy

Almost all authors who have reported the results of laparoscopic neurotomy in women with endometriosis have not classified the extent of the disease or have given cumulative results for all stages (Table I). Furthermore, as Sutton[33] observed, most published series "suffer from the disadvantage that they are entirely retrospective and uncontrolled and sceptics can quite reasonably argue that there is a massive placebo effect with this kind of symptom, especially if it is treated with the sort of high-tech wizardry that is implicit in laser beam procedures."

Feste[19] reported the resolution or marked improvement of dysmenorrhea in 72% of 32 women with endometriosis and chronic pelvic pain after excision of the implants and resection of the uterosacral ligaments by carbon dioxide laser. In 22% of the cases the symptoms did not change and in 6% they worsened. Using a similar technique, after 15 months of follow-up Davis[20] observed a considerable improvement in dysmenorrhea in 135 of 146 women (92%) and in dyspareunia in 103 of 109 women (94%) with endometriosis without significant differences in relation to the revised American Fertility Society classification stages.[34] An improvement of dysmenorrhea was observed by Daniell[21] in 75% of 80 patients with endometriosis 6 months after laparoscopic resection of the uterosacral ligaments performed with the potassium-titanium-phosphorus laser fiber. Sutton and Hill[22] used a carbon dioxide laser to section uterosacral ligaments and reported pain resolution in 70% of 187 women with endometriosis, dysmenorrhea, and dyspareunia after a variable follow-up of 1 to 6 years. Lichten and Bombard[35] have performed the only randomized, prospective, double-blind study designed to evaluate the efficacy of laparoscopic uterine nerve ablation by means of bipolar cautery and scissors transection. Unfortunately the presence of endometriosis was one of the exclusion criteria, but the trial is interesting for the objective evaluation of the potential pain relief afforded by the method. A relatively homogeneous group of women were selected who had severe or incapacitating dysmenorrhea and no demonstrable pelvic abnormality at laparoscopy and who were unresponsive to nonsteroidal antiinflammatory drugs and oral contraceptives prescribed concurrently. Coexisting psychiatric illness was evaluated with the Minnesota Multiphasic Personality Inventory, and those with an abnormal psychologic profile were excluded from the study. The remaining 21 patients were randomized to the group undergoing laparoscopic uterine nerve ablation or to the control group at the time of the diagnostic laparoscopy. Neither the patient nor the clinical psychologist who conducted the interview at follow-up was aware of the group to which the patient had been randomized. None of the patients in the control group reported relief from dysmenorrhea whereas 9 of the 11 patients (81%) who had laparoscopic uterine nerve ablation reported almost complete relief at 3 months and 5 of them had continued relief from dysmenorrhea 1 year after surgery. Evaluation of pain symptoms only 3 months after treatment is not very reliable,[36] whereas at 1 year after the operation only 45% of the women continued to report relief from dysmenorrhea.

Endometriosis rarely provokes exclusively midline pelvic pain that can definitely be lessened by paracervical or presacral denervation.[37] According to Daniell,[21] "It is important to counsel the patients with endometriosis and chronic pelvic pain carefully preoperatively and make certain that they are aware that success is not guaranteed in all cases." The uncertainties surrounding laparoscopic uterine nerve ablation were eloquently commented on by Vancaillie and Schenken[38]: "As with presacral neurectomy, the value of endoscopic neurotomy in relieving pain symptoms has not been established. Further, the complications of this procedure are unknown, but potentially great, because of the proximity of the ureter, rectosigmoid, and uterine vessels."

Very few judgments may be expressed on laparoscopic presacral neurectomy because only one observational report has been published to date.[23] After operation, among 25 patients, one required laparotomy because of retroperitoneal bleeding, four continued to experience the original adnexal discomfort, and two underwent subsequent hysterectomy with bilateral salpingo-oophorectomy because of failure to respond to conservative management. Only further studies will clarify whether presacral neurectomy is less risky at laparoscopy than at laparotomy or if it should be enumerated among the many operations that may be done "differently."[39]

Comment

> The validity of all evaluative studies of any therapeutic procedures depends on the level of certainty that exists on what would have happened without it.
>
> *T. Tulandi and N. Cherry*[40]
> 1989

Conservative surgery for treatment of endometriosis should undergo a rigorous and comprehensive reexamination regarding pain resolution. Most women with endometriosis report both lateral and midline pain. It has not been definitively demonstrated that presacral neurectomy, resection of the uterosacral ligaments, or both, which affect only the midline component, offer significantly better advantages than simple resection of the lesions as far as the general well-being of the women is concerned. Severe complications may follow presacral neurectomy,[17, 30] and this procedure should probably be performed in highly selected cases rather than in all women undergoing conservative surgery for treatment of endometriosis. The proposed laparoscopic technique[23] does not seem to reduce the risks and is probably untimely with respect to the current uncertainties on the real indications for the operation. Similar considerations also are relevant to paracervical denervation. Daniell[21] has recently written, "There have been at least two deaths in North America resulting from bleeding from uterosacral ligaments that occurred when these were transected with CO_2 laser energy. To date neither of these case reports have been published." Accepting without question a fixed chain of events—chronic pelvic pain, endometriosis, and neurectomy (presacral or paracervical)—may create unfounded therapeutic expectations. The mere presence of pelvic endometriosis may not cause symptoms.[41] Endometriosis has been found in 15% of pain-free women undergoing laparoscopic tubal sterilization.[42] Even when the uterus is believed to be the cause of chronic pelvic pain, hysterectomy does not improve symptoms in one woman out of four.[43] A multidisciplinary approach to evaluate thoroughly the psychic, neurologic, intestinal, and orthopedic components of the reported symptoms has been suggested[44, 45] and may be proposed to patients before surgery is performed for relief of pelvic pain presumed to be of endometriotic origin. However, even in women with true endometriotic pain, the large area over which lesions are often distributed on the peritoneum and pelvic organs must not be forgotten.[37, 46] As advised by other authors,[18] a multicenter trial in which the eligibility criteria and surgical procedures are precisely described and carefully monitored would be opportune. So far, there are inadequate data to confirm or refute the effectiveness of presacral neurectomy and amputation of the uterosacral ligaments in the treatment of chronic pelvic pain associated with endometriosis.

A last comment concerns the instruments to be used in laparotomy and laparoscopic denervation. Soderstrom[17] maintains that, however delivered, "a watt is a watt," and that to date the clinical studies touting the benefits of laser over electrosurgery have not been convincing. Leaving aside the theoretic advantages that still have not been definitely proved, laser treatment, especially if endoscopic, is fashionable to the extent of figuring in the most widely circulated international magazines[48-50] that show laparoscopists armed with lasers proclaiming that "In 20 years, major abdominal surgery will be nearly extinct."[50] In the meantime the "traditional" surgeon can console himself with the words of Illingworth[51]: "That which is most up to date and new is not necessarily the best."

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BASIC SCIENCE SECTION

Inhibin subunits in human placenta: Localization and messenger ribonucleic acid levels during pregnancy

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This study describes the difference in distribution and levels of inhibin α, βA- and βB-subunit messenger ribonucleic acids in human placenta during pregnancy. Northern blot analysis indicated that inhibin α messenger ribonucleic acid is present in placental extracts collected at the early stage of gestation. Hybridization to inhibin βA messenger ribonucleic acid was detected in the first trimester but in much lower levels. However, the intensity of the hybridization signal for inhibin α- and βA-subunit messenger ribonucleic acids was greater in extracts prepared from term placentas than in those from the first or second trimester of pregnancy. Low levels of inhibin βB-subunit messenger ribonucleic acid were observed only in extracts prepared from term placenta. At both early stage and term gestation trophoblast cells showed a positive fluorescent signal with the inhibin α-, βA- and βB-subunit–specific antisera. However, whereas inhibin α-subunit was localized in the cytotrophoblast, inhibin βB-subunit immunoreactivity was observed in the syncytial layer of the villi, and inhibin βA-subunit was widely distributed. The different distribution of immunoreactive inhibin subunits was confirmed by in situ hybridization, showing the different localizations of the inhibin messenger ribonucleic acids. These results showed that (1) human placenta produces the inhibin α- and βA-subunits as early as the first trimester of pregnancy, (2) messenger ribonucleic acid levels for each of the three inhibin subunits are highest at term, and (3) immunoreactive inhibin subunits are localized differently in placental villi. (AM J OBSTET GYNECOL 1991;165:750-8.)

Key words: Inhibin, activin, placenta, proteins, growth factors, pregnancy

Two new hormones, inhibin and activin, are produced by human placentas.[1-4] Indeed, placenta seems the major source of the high levels of circulating inhibin-like immunoreactivity in pregnant women.[5-7] Moreover, inhibin and activin modulate human chorionic gonadotropin (hCG) and progesterone release, suggesting a local paracrine or autocrine effect.[8,9]

Inhibin is a disulfide-linked heterodimer composed of an α-subunit and one of two distinct β-subunits (βA or βB), whose major biologic role is to decrease follicle-stimulating hormone release from pituitary gland.[10,11] The combination of two β-subunits (βA and βA, βA and βB, βB and βB) originates a dimer named activin, which increases pituitary follicle-stimulating hormone release.[10,11] However, there are not yet convincing methods to evaluate which forms of inhibins or activins are present in human placenta.

Earlier studies have described the presence of inhibin α-subunit immunoreactive material, in human term placenta[3] as well as inhibin α- and βA-subunit messenger ribonucleic acid (mRNA).[1,4] Rat placenta, on the other hand, produces mRNA encoding inhibin α-, βA- and βB-subunits.[12] However, no data on the possible different expression of the inhibin subunits in human placenta during pregnancy or on the localization of the inhibin β-subunits within placental villi are currently available. The goal of this study was to evaluate the expression of the respective inhibin subunit mRNAs and the cellular localization of these subunits in human placentas at various stages of gestation.

Material and methods

Tissue preparation. Placental tissue was collected from elective aspiration termination (8 to 10 weeks of

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pregnancy, $n = 13$), from therapeutic abortion (18 weeks of pregnancy, $n = 2$), and from elective cesarean section (39 to 40 weeks of pregnancy, $n = 7$). For specimens collected at an early stage of gestation, the placental villi were distinguished from the decidual tissue according to the external appearances, with the use of a dissecting microscope. To confirm the absence of decidual or fetal tissues, a histologic evaluation of the specimens collected was carried out in parallel. The placental tissue from the second trimester and at term was collected within the central structure of the cotyledons, avoiding the basal plate. According to the following procedures, placental tissue was subdivided in three aliquots: (1) Some tissue was immediately placed in guanidine isothiocyanate and treated for ribonucleic acid (RNA) extraction and subsequent Northern blot analysis; (2) some trophoblast was immediately frozen in liquid nitrogen for immunohistochemistry; (3) part of the trophoblast was fixed in 4% paraformaldehyde for 8 hours before freezing for the in situ hybridization studies.

Northern blot analysis. The collected tissue samples were immediately homogenized in 4 mol/L guanidinium isothiocyanate, 25 mmol/L sodium citrate (pH 7), 0.5% sarcosyl, and 0.1 mol/L 2-mercaptoethanol, and total RNA was isolated.[13] Placental total RNA samples were pooled according to gestational age, and poly(A) mRNA enrichment was subsequently obtained by oligo(dT)-cellulose column chromatography. Total poly(a) mRNAs were quantified by ultraviolet absorptions at 260 nm, size fractionated by electrophoresis on 1.5% formaldehyde and agarose gel, and transferred to gene screen membranes (New England Nuclear, Boston). Three identical series of placental poly(a) mRNAs (5 μg; each sample from a different trimester of gestation) were electrophoresed and blotted on the filters. The experiments were replicated three times.

The complementary deoxyribonucleic acid (cDNA) containing human inhibin α, βA, and βB and γ-actin sequences were excised from the plasmid vector and purified by agarose gel electrophoresis. Deoxyribonucleic acid (DNA) labeled with DNA polymerase (Klenow Enzyme, Boehringer Mannheim, Mannheim, Federal Republic of Germany) in the presence of deoxyadenosine triphosphate and ^{32}P-a-deoxycytidine, with the random phosphate 32–α-priming technique (specific activity of 2 to 4 × 10^8 cpm/μg DNA was usually obtained).[13]

Blots were prehybridized in 4× SSPE (1× SSPE is 0.15 mol/L sodium chloride, 10 mmol/L sodium biphosphate, and 1 mmol/L ethylenediaminetetraacetate), 10× Denhardt's solution (1× Denhardt's solution is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 0.1% sodium dodecyl sulfate, 0.2 mg/ml sonicated salmon sperm DNA, and 0.2 mg/ml yeast-extracted tRNA for 2 hours at 65° C. After addition of the labeled human inhibin α-, βA-, and βB-subunit probes (10^7 counts/min/ml), three independent hybridizations were carried out for 15 to 20 hours at 65° C. The filters were then washed in 0.2× SSPE and 0.5% sodium dodecyl sulfate at 65° C and exposed to Kodak X-AR film with an intensifying screen at −40° C for 48 hours. As an internal control, the filters also were hybridized with human γ-actin cDNA probe. Results shown are representative of three separate experiments. Autoradiograms were scanned with a laser densitometer (Ultrascan XL, LKB, Uppsala). The integrated values for the hybridization signal of inhibin α-, βA-, and βB-subunit mRNA were corrected according to the specific activity of each probe and were expressed as arbitrary densitometric units.

Immunohistochemistry. Serial sections (10 μm) were cut on a cryostat (Leitz, Rockleigh, N.J.) and mounted on glass slides coated with chrome alum and gelatin to prevent their detachment during the incubations. The slides were then immersed in 4% paraformaldehyde in phosphate-buffered saline solution, pH 7.6 (15 minutes), washed in 0.1 mol/L phosphate-buffered saline solution (10 minutes), and incubated in the presence of the antiserum (diluted in 0.3% Triton X100 phosphate-buffered saline solution) for 18 hours at 4° C in a humid atmosphere. These highly specific antisera were used: (1) anti-inhibin α (1-26)-Gly-Tyr (dilution 1:400), (2) anti-inhibin βA (81-113) sodium hydroxide (dilution 1:1000), and (3) anti-inhibin βB (81-112) sodium hydroxide (dilution 1:500). These were affinity-purified polyclonal antisera raised in rabbit against porcine moieties. Their characteristics have been described in detail previously.[13] No cross-reaction between βA and βB antisera was observed. After rinsing in phosphate-buffered saline solution (3 × 10 minutes), the sections were incubated in the presence of the second antiserum (fluorescein isothiocyanate-conjugated swine antirabbit immunoglobulin) (Dako, Copenhagen) in 0.3% Triton X100 phosphate-buffered saline solution (30 minutes at 37° C). Once rinsed in phosphate-buffered saline solution, the sections were dried out, mounted in glycerol–phosphate-buffered saline solution (3:1 vol/vol) and examined with a fluorescent photomicroscope (Nikon Microphot-FXA, Tokyo). Control sections were preadsorbed with the synthetic immunogens or in the absence of the first antiserum.[13]

In situ hybridization. Antisense ^{3}sulfur 35–labeled RNA probes specific for the human inhibin α-, βA, or βB-subunit were generated by transcription for the SP6 or T7 promoters of linearized pGEM4 subclones, each to a specific activity of 5 × 10^7 to 1 × 10^8 counts/min/μg. The human inhibin RNA probe complementary to the α-subunit comprised 546 nucleotides, 353 corresponding to the mature portion of the

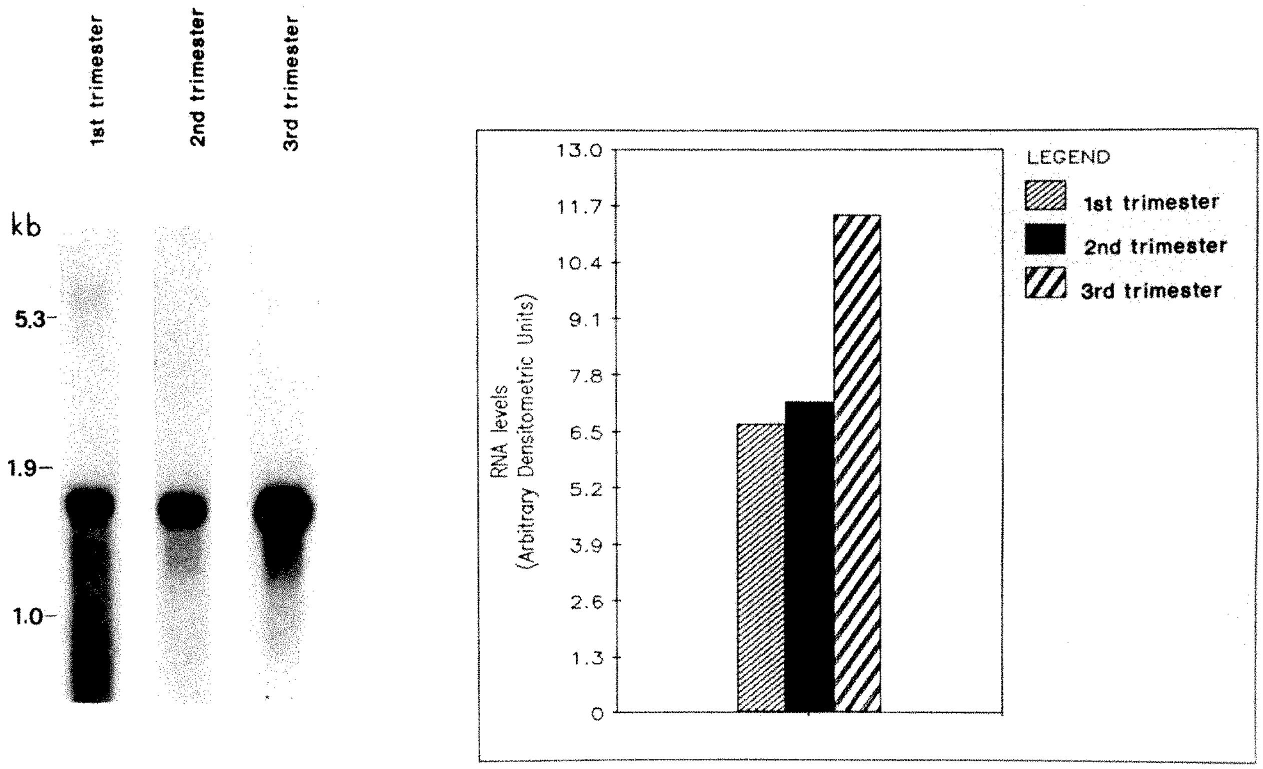

Fig. 1. Northern blot analysis of inhibin α-subunit mRNA in placental tissue at various gestational ages. Subunit mRNA is 1.5 kb. Quantitative evaluation of mRNA levels after hybridization revealed highest levels at term. For each lane 5 μg of poly(A) mRNA was electrophoresed.

molecule and 193 trailing into the 3′-untranslated region. The inhibin βA and βB mRNA probes were 338 and 320 nucleotides in length, respectively. The complementary RNA probe specific for the βA-subunit included 222 nucleotides spanning the mature region and 116 spanning the 3′-end untranslated region. The probe complementary to βB mRNA contained nucleotides from the 3′-end. Background levels were determined with a sense mRNA probe for the inhibin α-subunit (353 nucleotides). Differences in hybridization patterns suggest that the βB mRNA probe does not cross hybridize with βA-subunit mRNA.[11]

Inhibin-subunit mRNA was detected by the method previously described.[11] Frozen tissue was cut on a cryostat (20 μm) and mounted on poly-L-lysine–coated slides. Sections were incubated at room temperature for 30 minutes in the same fixative used for perfusion, rinsed, incubated at room temperature for 10 minutes in 0.5% Triton X100 in 0.1 mol/L Tris, 0.05 mol/L ethylenediametetraacetate, pH 8.0 buffer, and then rinsed. Dehydrated sections were incubated for 16 hours at 63° C with labeled RNA probes. After ribonuclease I (20 μg/ml) treatment, desalted sections were washed in 15 mmol/L sodium chloride/1.5 mmol/L sodium chloride with 1 mmol/L dithiothreitol at 55° C for 30 minutes. After successive alcohol dehydration, the sections were exposed to β-max hyperfilm (Amersham, Arlington Heights, Ill.) for 1 or up to 3 days. Slides were then defatted, dipped in nuclear tract emulsion (NTB-2, Kodak), and exposed for 18 days. After development (Kodak D19) and fixation (Kodak, Ektaflo), slides were stained with hematoxylin and eosin. Photographs were taken with Ilford XP1 film.

Results

Expression of inhibin subunit mRNA in placental tissue. A considerable difference in the degree of expression of the inhibin α-, βA-, and βB-subunits was observed among the tissue samples collected at the various trimesters of gestation. Placental tissue at first trimester of pregnancy expressed intense inhibin α and slight βA (Figs. 1 and 2) but not inhibin βB-subunit mRNA. The mRNA levels of the α- and βA-subunits in the placental tissue collected in the second trimester

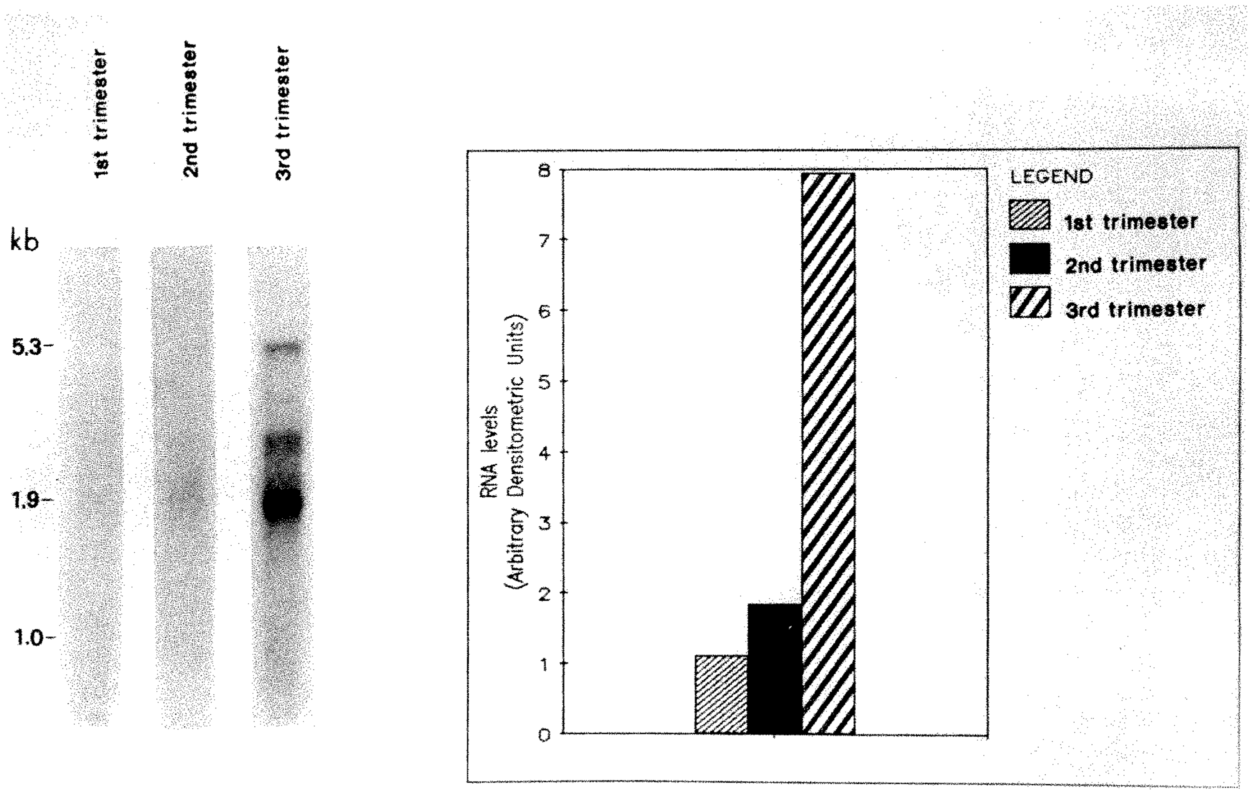

Fig. 2. Northern blot analysis of inhibin βA-subunit mRNA in placental tissue at various gestational ages. Three main species of βA mRNA were found: 5.3 kb, 2.9 kb, and 1.9 kb in size. In all placental samples 1.9 kb mRNA was most intense, suggesting that 5.3 and 2.9 kb mRNA species could present partial splicing products.[4, 11] Levels of mRNA were highest at term. For each lane 5 μg of poly(A) mRNA was electrophoresed.

were similar to those found in the first-trimester placentas. Placental tissue at term contained the highest levels of α- and βA-subunit mRNA (Figs. 1 and 2). Low levels of inhibin βB-subunit mRNA were observed only in placental tissue at term. The intensity of the hybridization signal for the βB-subunit was much lower and required a longer exposure time (data not shown). Supporting the specificity of the gestational changes of inhibin subunit expression, γ-actin mRNA levels did not vary with gestational age (Fig. 3).

Localization of inhibin subunits in placental tissue. Considerable variations were observed in the intensity and distribution of immunoreactive material (α, βA, and βB) between early and term gestation. In early pregnancy most immunoreactive inhibin α-, βA-, and βB-subunits were localized in trophoblast cells. However, positive immunostaining also was localized in the core of the villi (mesenchymal cells). An intense positive nuclear staining was observed in some preparations. In the first trimester inhibin α-subunit was found in cytotrophoblast columns or diffusely in the inner part of the villi (Fig. 4, *A* and *B*). The staining for inhibin βB-subunit was more intense and localized both in the inner (cytotrophoblast and villous core cells) and in the outer (syncytiotrophoblast) layers of the villi (Fig. 5, *A* and *B*). Immunoreactive inhibin βB-subunit was detected in the outer syncytial layer (Fig. 6, *A* and *B*). Preadsorption with the respective immunogens completely abolished the specific patterns of each antiserum (Figs. 4, 5, and 6, *C*). Similarly, the control sections incubated with the second antibody conjugate did not show any positive signal.

Staining was more intense in placental tissue collected at term than in tissue from early pregnancy for all three inhibin subunits. Again, the cellular distribution of the various inhibin subunits at term followed different patterns. Confirming our previous observation,[3] immunoreactive inhibin α-subunit was mostly localized in the cytotrophoblast cells and a light signal was observed in the syncytiotrophoblast (data not shown). An intense signal of immunoreactive inhibin βA was found in both cytotrophoblast and syncytial cells (Fig. 7, *A*), whereas

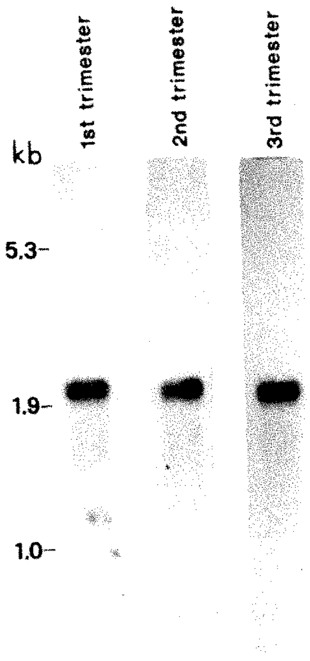

Fig. 3. Northern blot analysis of placental tissue at various gestational age with γ-actin cDNA probe. For each lane 5 μg of poly(A) mRNA was electrophoresed.

immunoreactive inhibin βB fluorescence was detected in the external layer of the villi (Fig. 7, *C*). Preabsorption with the respective immunogens competed efficiently for endogenous subunits, as recognized by the specific antisera (Fig. 7, *B* and *D*).

The in situ hybridization analysis revealed that inhibin subunit mRNA signals followed a different pattern of distribution as shown by immunoreactive proteins. The βA-subunit mRNA was the most distributed; signals were found both in the external layer and in the inner part of the villi (Fig. 8). The α-subunit mRNA was localized within the structure of the villi and a slight signal of βB-subunit mRNA in the syncytial cells (Fig. 8). Background levels of signals were partially detected in the various control slices, but grain density was lower than in experimental photomicrographs (Fig. 8).

Comment

These results show that human placentas produce inhibin α-, βA-, and βB-subunits with a gestation-related profile, the highest levels being observed at term. Moreover, while confirming that inhibin α-subunit is localized in cytotrophoblast and in some of the villous core cells, our data showed that in the placenta inhibin βA- and βB-subunits are subject to a different distribution pattern. Indeed, inhibin βA-subunit is distributed both in the inner and in the outer part of the placental villi, and the inhibin βB-subunit is almost exclusively localized in the syncytial cells.

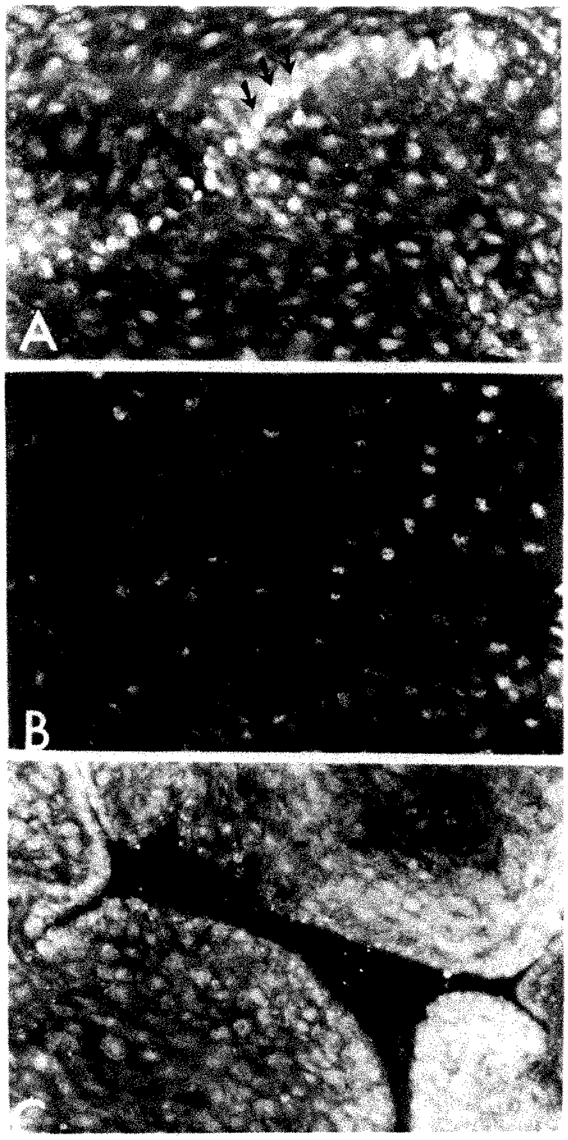

Fig. 4. Indirect immunofluorescence localization of inhibin α-subunit in human placental tissue during first trimester of pregnancy (**A** and **B**). **C,** Control section after preabsorbtion with inhibin α-subunit immunogen. (Original magnification × 160.)

Human placenta is one of the major examples of extragonadal expression of inhibin subunits. As previously suggested, the relative abundance of each inhibin mRNA may be indicative of which inhibin (αβ) or activin (ββ) dimer is produced in various tissues.[10, 15] The level of expression of the three mRNA inhibin subunits in placental tissue differed throughout pregnancy, leading us to suggest that the production of inhibin and activin changes during gestation. The gestation-related increase of inhibin α-subunit mRNA levels may reflect an increase in the synthesis of inhibin

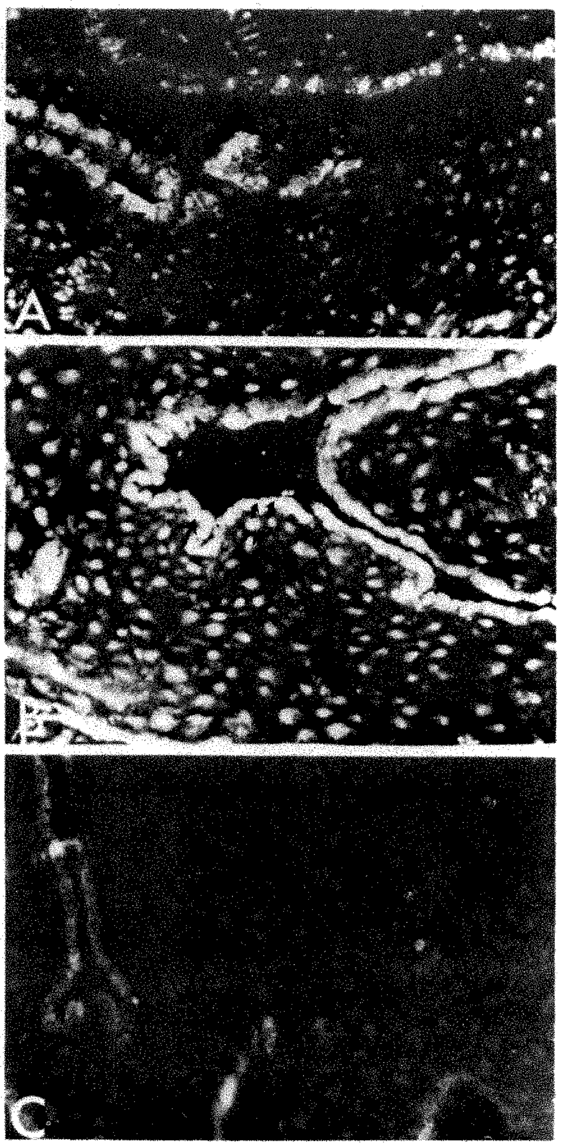

Fig. 5. Indirect immunofluorescence localization of inhibin βA-subunit in human placental tissue during first trimester of pregnancy (**A** and **B**). Immunoreactive inhibin βA-subunit material is observed within and surrounding placental villi. **C,** Control section after preadsorption with βA-subunit immunogen. (Original magnification × 160.)

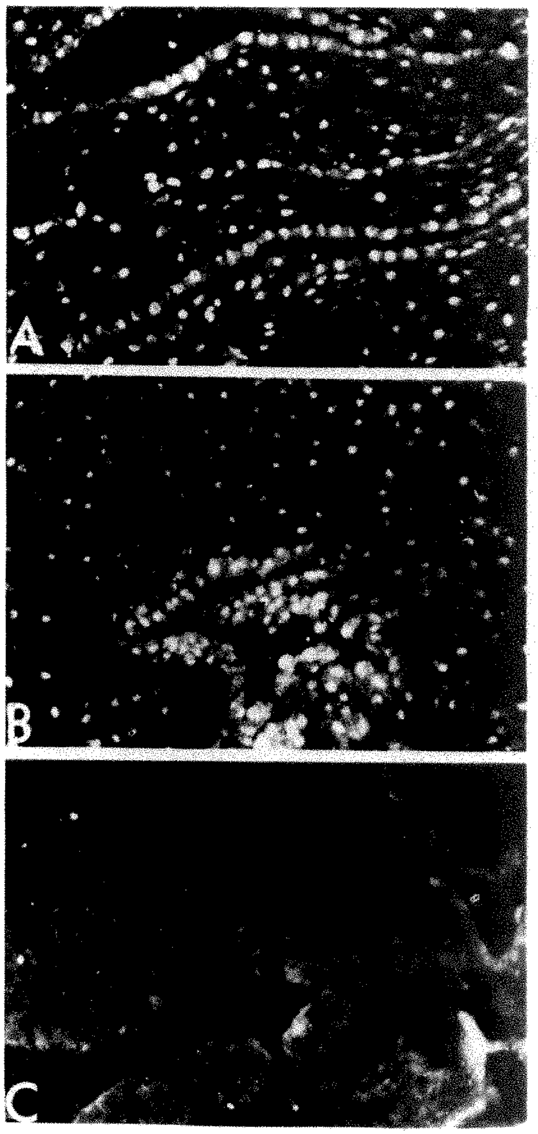

Fig. 6. Indirect immunofluorescence localization of inhibin βB-subunit in human placental tissue during first trimester of pregnancy. Syncytial cells are labeled by inhibin βB–specific antiserum. **C,** Control section after preadsorption with inhibin βB immunogen. (Original magnification × 160.)

during pregnancy. This hypothesis is supported by the increased levels of maternal plasma inhibin, as described in healthy pregnant women.[2] The synthesis of several placental hormones increases during gestation,[16] and a possible correlation between the inhibin subunit level changes and the other hormonal changes may be hypothesized.

The presence of both inhibin α- and βA-subunit mRNA in first-trimester placenta suggests that inhibin A (αβA) or activin A (βAβA) may be produced in the early stages of gestation. Small amounts of inhibin βB-subunit mRNA have been detected only in placentas collected at term, suggesting a late and low expression of the gene encoding the βB-subunit. However, immunoreactive inhibin βB-fluorescent material was observed in placental tissue collected in early pregnancy. The discrepancy between the undetectable or low βB-subunit mRNA signal and the presence of immunoreactive βB in the trophoblast tissue may be explained by the different sensitivity of the techniques involved. The Northern blot and the in situ hybridization analyses may not detect the very low levels of mRNA, whereas antisera are sensitive enough to detect small amounts of the corresponding protein. Another pos-

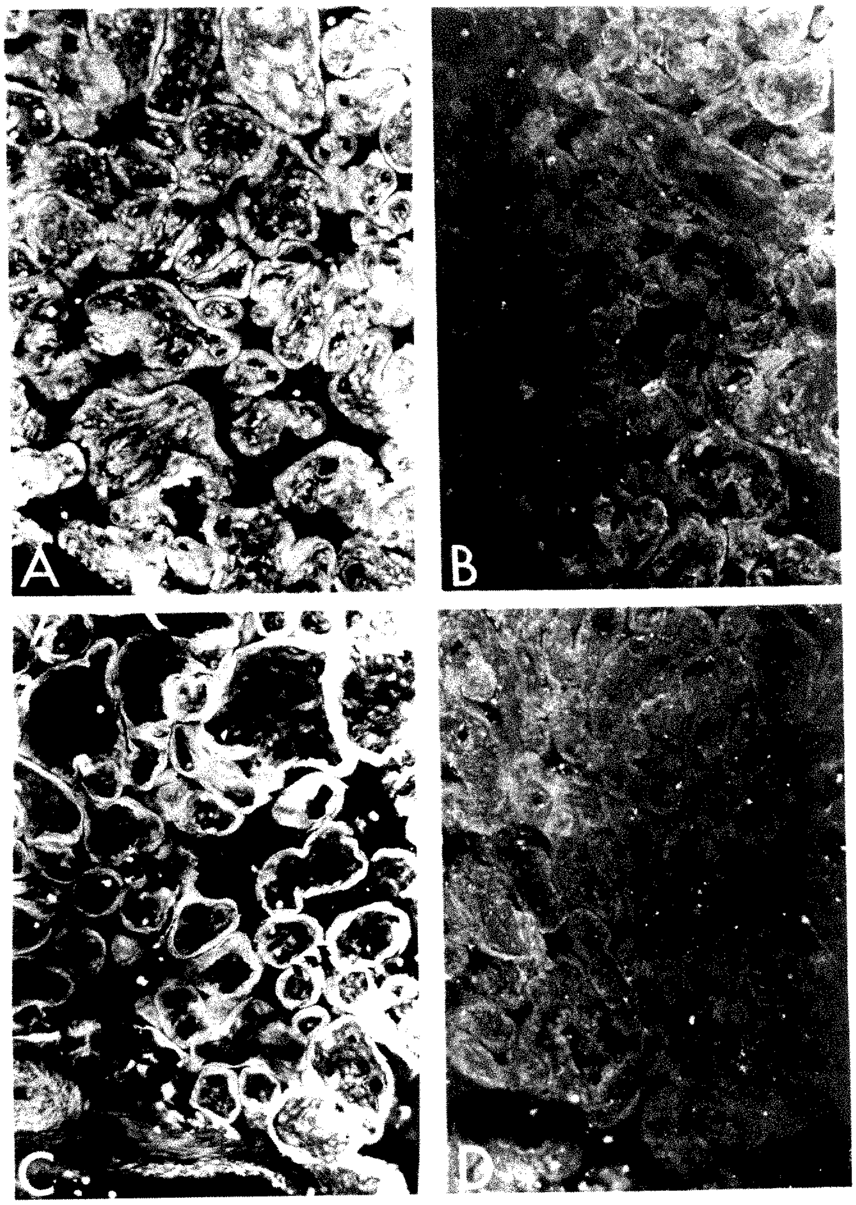

Fig. 7. Indirect immunofluorescence localization of inhibin subunits in human placental tissue at term. **A** shows widespread localization of immunoreactive inhibin βA-subunit material in inner and outer layers of placental villi. **C,** Syncytial cells positively staining inhibin βB-subunit. **B** and **D** show control section after preadsorption with βA and βB immunogens, respectively. (Original magnification ×210.)

sibility is that the βB-subunit mRNA in the syncytiotrophoblast cells may be subjected to rapid turnover. Alternatively, the immunoreactive inhibin βB-subunit present in the syncytial cells could be bound or sequestered from another source; only a limited amount results from a local synthesis. The same type of discrepancy between positive immunostaining and lack of detection of the βB mRNA signal has been described recently in rat testis.[14] The low signal in placental tissue is in contrast with the high levels of inhibin βB-subunit mRNA detected in human decidua throughout pregnancy.[13]

The different cellular localization of the respective mRNA inhibin subunits suggests that β-heterodimers and βB-homodimers, i.e., inhibin and activin, may have different sites of production. The presence of both α- and βA-subunits in the inner part of the villi could imply that inhibin A (α βA) and activin A (βAβA) may be produced by cytotrophoblast. The presence of βA- and βB-subunits in the outer part of the villi suggests that syncytial cells may synthesize the activins (βAβA, βAβB, βBβB).[10, 15] These results describing the distribution pattern of the inhibin subunits in human trophoblast resemble those previously described for an-

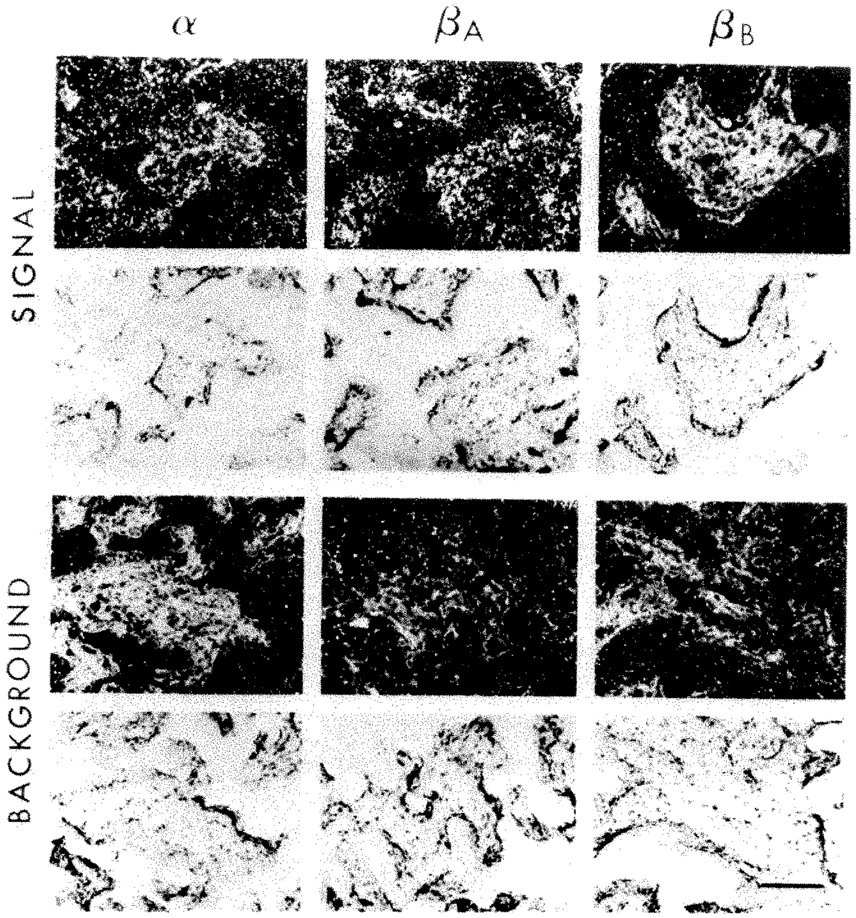

Fig. 8. Dark-field and bright-field photomicrographs show distribution of inhibin α, βA-, and βB-subunit mRNA, by in situ hybridization, in human placenta at term. α-Subunit mRNA is localized in centrally situated cytotrophoblast layer *(arrow)* of placenta villi, whereas βA-subunit mRNA is found in both inner and outer layers of villi. βB-Subunit mRNA signal is very low and is located in outer part of villi. Control mRNA photomicrographs show background levels of signals with labeled sense strand mRNA probe for each inhibin subunit. *Solid bar* represents 20 μm.

other typical placental glycoprotein of two subunits, hCG. In fact, whereas the hCG α-subunit mRNA is expressed in both cytotrophoblast and syncytial cells, the hCG β-subunit is expressed only in the syncytiotrophoblast.[16]

Therefore inhibin and activin may be included among the placental protein hormones, and multiple targets could be hypothesized. In defining a putative role of placental inhibin glycoproteins, both paracrine or autocrine and endocrine effects may be suggested. Indeed, gonadotropin-releasing hormone, progesterone, and gonadotropin-releasing hormone–induced hCG release from cultured placental cells is increased in the presence of activin and decreased after the exposure to inhibin.[8, 9] Moreover, the presence of placental inhibins in the maternal or fetal circulation[2, 3, 5, 7] suggests that maternal or fetal functions also may represent a target for those hormones or growth factors.[16]

In conclusion, our data revealed that human placenta all throughout pregnancy synthesizes the inhibin α-, βA-, and βB-subunits, indicating that the highest mRNA levels occur at term and that they have different cellular distributions. These results emphasize human placenta as one of the most important extragonadal sources of inhibin and activin.

We wish to thank Pamela Mellon, PhD (Salk Institute), for providing the γ-actin cDNA probe and Renata Battini for evaluating the mRNA scanning densitometry. Moreover, we acknowledge the help of Maria Cristina Galassi, Anna Mancini, Joan Vaughan, Sandra Guerra, and Beth Coyne.

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Circadian rhythms of heart rate and mean arterial pressure in chronically instrumented pregnant rats

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With the use of a chronic preparation to directly monitor blood pressure and heart rate in pregnant rats, continuous data were obtained over the last half of gestation in normotensive rats. Over this time span, the animals showed significant decreases in blood pressure and increases in heart rate. Heart rate exhibited marked and consistent circadian rhythmicity with peaks occurring near the midportion of the dark phase of the 24-hour cycle. Blood pressure rhythms were less prominent and peaked later. The trends observed in blood pressure and heart rate over gestation suggest that the pregnant rat is a useful model for studying the cardiovascular effects of pregnancy. (AM J OBSTET GYNECOL 1991;165:758-63.)

Key words: Rat pregnancy, circadian rhythms, blood pressure, heart rate

In many mammals, including humans,[1-4] cardiovascular functions exhibit circadian rhythmicity. These rhythms, which have been described by many investigators,[1-6] are known to be influenced by the light-dark and rest-activity cycles. Because pregnancy imposes strong and progressive changes in maternal cardiovascular function in many species, including rodents,[7-10] we chose to examine the effects of pregnancy on circadian rhythms of heart rate and blood pressure in normotensive rats.

Although nonpregnant rats are known to demonstrate prominent circadian rhythmicity in many cardiovascular functions,[4-6] few studies exist to examine how these rhythms are influenced by gestation. Most previous investigations of cardiovascular rhythms in rats have used indirect (tail cuff blood pressure mea-

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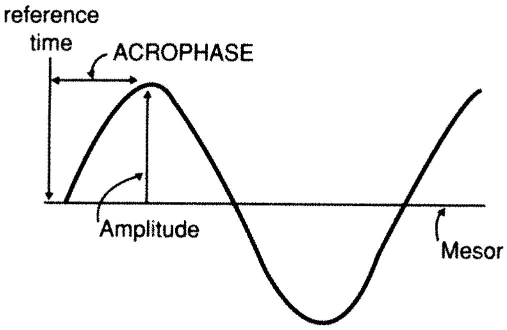

Fig. 1. Several measurable parameters of rhythmic behavior are shown: *Acrophase*, lag between reference time (12 midnight in 24-hour day) and time of rhythm's crest; *amplitude*, half of total predictable change in rhythm, *mesor* (Midline Estimating Statistic of Rhythm), average value of cosine curve fitted to data (usually halfway point between peaks and troughs of cosine curve).

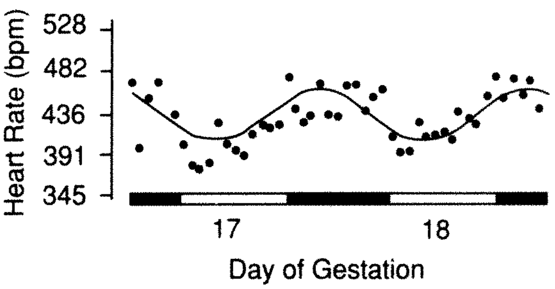

Fig. 2. 3M-63: Typical 48-hour plot of heart rate in one pregnant rat.

surements) methods to obtain data.[4-6] However, the restraint and warming involved in tail cuff measurements produce marked elevations in heart rate and (in some cases) blood pressure and are likely to obscure circadian rhythms.[11] Improvement measurement of cardiovascular functions can be achieved by chronic arterial cannulation. Reports published by several groups of investigators have described direct arterial blood pressure measurements in acute and chronic rat preparations.[10-13] Pan et al.[10] have described the use of this preparation in near-term pregnany rats. We are not aware of previous studies providing longitudinal data over a substantial portion of rat gestation. The object of this study was to develop a chronic model and to study circadian rhythms of cardiovascular functions longitudinally in pregnant rats throughout gestation. The hypothesis to be tested was whether circadian rhythms of blood pressure and heart rate were present.

Methods

Nine outbred Sprague-Dawley rats (Zelianople, Pa.) were used in the experiment. All facilities and procedures were approved by our Animal Care and Use Committee. One week of acclimatization to the 12-hours-light/12-hours-dark cycle was allowed before surgery. Surgery was performed on the eleventh day of gestation. The surgical procedure was an adaptation from the methods of Chiueh et al.[11] and Fejes-Toth et al.[14] The rats were anesthetized with ketamine hydrochloride (100 mg/ml) and xylazine (20 mg/ml), which were mixed in a 3:1 ratio and were administered intramuscularly (0.10 ml/100 gm body weight). The ventral surface of the tail and the nape of the neck were shaved and cleansed with 70% ethanol, a 2 cm incision was made in the base of the tail, and the middle caudal artery was dissected from its fascial canal. A 4-0 silk ligature was used to secure the distal artery. The vessel was incised, and a polyethylene catheter (PE-10 tip, PE-90 body) was inserted 8.5 cm through the artery to the abdominal aorta; the tip was placed just below the level of the renal arteries. The cannula was secured with 4-0 silk suture. The body of the cannula was tunneled subcutaneously via a trochar along the dorsal surface to exit centrally in the prepared area at the nape of the neck. The tail incision was closed withd 3-0 Vicryl (polyglycolic acid) suture. Steri-strips (3M Co., Minneapolis) were wrapped around the tail to prevent the animal from disturbing the surgical site and to provide wound support.

The neck incision was extended 1 cm parallel to the rat spine, and the skin was undermined. A 2 cm round piece of sterile Dacron netting was fixed with cyanoacrylate to the muscle. A Teflon button was secured with 1-0 Vicryl to the skin with transfixation via the subcutaneous Dacron netting, and a flexible stainless steel spring was attached to the button with Allen screws. Di-Trim (400 mg sulfadiazine and 80 mg/ml trimethoprim) was given intramuscularly (0.1 ml/100 mg) at the end of the surgical procedure. The rat was placed in a specially modified cage[15] and the cannula was secured to a fluid swivel (Instech Corp., Plymouth Meeting, Pa.), which permitted free movement of the animal without undue restraint. Cannula patency was maintained by a slow infusion (2 to 4 ml/24 hr) of heparinized saline solution (3.0 IU/ml) from a multichannel peristaltic pump (Ismatek SA, Zurich). Rats were fed Purina Rat Chow (Ralston Purina Co., St. Louis) and water as desired. The environmental temperature was maintained between 23° and 25° C. The light-dark cycle consisted of 12 hours of light and 12 hours of darkness; lights were turned on at 6 AM. The digitized, pulsatile output of a pressure transducer was sampled 2400 times per minute by an IBM-compatible computer. Systolic blood pressure, diastolic blood pressure, mean arterial pressure (MAP), and heart rate, which was computed from the arterial pressure wave, were averaged each minute and stored; 1-hour averages and their measures of variation were simulta-

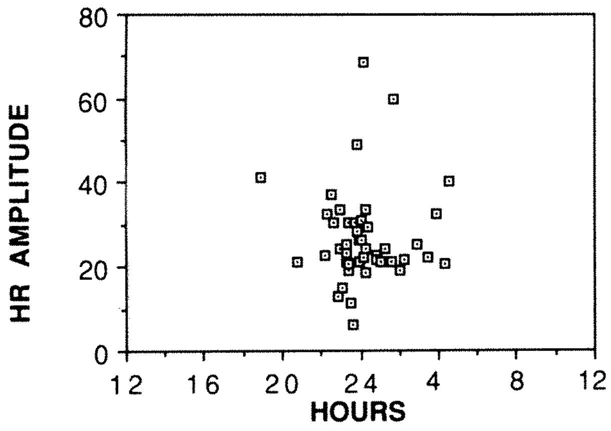

Fig. 3. Timing in clock hours of all heart rate peaks (acrophases) plotted by heights of peaks (amplitudes) in beats per minute. Data from all statistically significant single cosinor analyses of all animals are included.

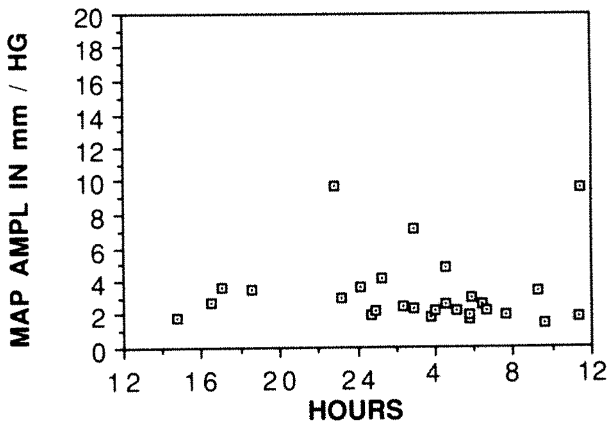

Fig. 5. Timing in clock hours of all MAP peaks (acrophases) plotted by heights of peaks in millimeters of mercury (amplitudes). Data from all statistically significant single cosinor analyses of all animals are included.

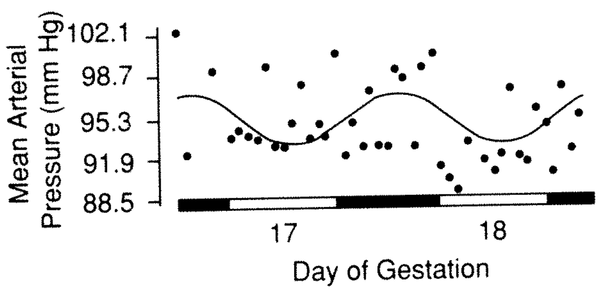

Fig. 4. 3M-63: Typical 48-hour plot of mean arterial pressure in one pregnant rat.

neously printed out and stored on a floppy disk. Six-hour and 24-hour averages also were stored and printed. The program used was written by Dr. Dennis Wolf, Department of Physiology, Creighton University Medical School, Omaha.

Data analysis. Information from these chronically instrumented animals constituted a large and dense base of blood pressure and heart rate data collected over the last half of gestation. Over the course of the study, 2376 hourly intervals were examined, each of which was derived from 2400 samples for each minute of recording.

These data were examined by consecutive 48-hour epochs by individual animal and by group mean analysis of all animals for each 48-hour time span of gestation. The animals were allowed to recover from anesthesia and surgery. By day 13, when stable heart rates were seen, data analysis was begun. This analysis was continued through day 20 but was stopped approximately 36 hours before the onset of labor, because the process of parturition introduces other effects on blood pressure and heart rate. The presence of circadian rhythmicity in MAP and heart rate in the data was examined by single cosinor analysis.[16, 17] This technique uses the fitting of a cosine curve to the data by means of least-squares regression. A period of 24 hours was chosen as the cycle length to be examined. An F statistic was then used to test the zero-amplitude hypothesis for a circadian rhythm. For statistically validated rhythms ($p < 0.05$), the following parameters (and their 95% confidence intervals) were estimated: the mesor (rhythm-adjusted mean), the amplitude (half the total extent of the predictable change), and the acrophase (timing of the cosine wave peak). A depiction and definitions of these rhythmic parameters are shown in (Fig. 1).

In addition to this analysis of individual time epochs, the group mean cosinor technique was used to analyze grouped data from all pregnant animals at each successive 48-hour time epoch of gestation to evaluate the presence of population circadian rhythmicity.

The program for single and group mean cosinor analysis on a PC-compatible computer was provided by Dr. Bartholomew Hsi, University of Texas Health Science, Houston.

Results

Heart rate. A 48-hour plot of representative heart rate data is shown in Fig. 2. Single cosinor analysis demonstrated statistically significant circadian rhythms of heart rate in 34 of the 36 epochs examined. In those time series that exhibited rhythmicity, mean amplitude of the circadian rhythms was 24.72 beats/min (range 5.1 to 68.8). The acrophases or peak times of these rhythms ranged from 7 PM to 5 AM. Fig. 3 shows the plot of acrophase and amplitude of the epochs demonstrating circadian rhythmicity. This graph indicates a tight grouping of the peak times with most peak times occurring between 10 PM and 2 AM.

The group mean cosinor technique analyzed the presence of circadian rhythmicity from all animals that demonstrated statistically significant rhythms during a

Table I. Group mean cosinor analysis of heart rate

Days' gestation	*Mesor*		*Amplitude*		*Acrophase*		*n*	*p Value*
	Mean	*95% Confidence interval*	*Mean*	*95% Confidence interval*	*Mean*	*95% Confidence interval*		
14-15	393.6	377.6-409.5	21.6	10.3-32.9	10:39 PM	9:20 PM–12:46 AM	7	0.004
16-17	393.1	382.9-403.3	21.0	16.1-25.9	11:22 PM	10:31 PM–12:09 AM	9	<0.001
18-19	409.2	396.1-422.2	24.6	18.2-31.1	11:32 PM	10:17 PM–1:42 AM	9	<0.001
20-21	417.4	404.2-430.6	27.1	20.5-33.6	10:59 PM	8:37 PM–2:18 AM	9	<0.001

Group mean value and 95% confidence intervals are shown for heart rate mesor (mean), amplitude (height of rhythm peak above baseline), and acrophase (timing of peak). Number of animals that exhibited significant rhythmicity of heart rate by single cosinor analysis is indicated.

particular 48-hour time epoch from days 14 and 15 to days 20 and 21. The results for heart rate are shown in Table I. Statistically significant circadian rhythms of heart rate are shown in each period of pregnancy for the pooled data. The timing of the peak heart rate (acrophase) ranged from 10:39 PM to 11:32 AM.

Blood pressure. A 48-hour plot of representative MAP data is shown in Fig. 4. Single cosinor analysis of mean arterial pressure demonstrated statistically significant rhythms in 24 of 36 epochs. In these 24 time series the mean amplitude of the rhythms was 2.92 (range, 0.51 to 8.0). During the first two sampling periods acrophases of MAP were variable and occurred at almost all hours of the 24-hour circadian time period. In the last two sampling times (days 18 to 19 and 20 to 21) the times of blood pressure rhythm peaks were much more consistent and clustered around 0. Fig. 5 plots MAP acrophase and amplitude and shows the wide range of dispersion of peak times and relatively low amplitudes of these MAP rhythms. Group mean cosinor analysis of MAP is shown in Table II. Statistically significant circadian rhythms could not be demonstrated in the two epochs 14 to 15 and 16 to 17 but were found for days 18 to 19 and 20 to 21. The mean or mesor of MAP decreased during gestation from 99.8 mm Hg at midgestation to 92.2 mm Hg at term (F ratio 10.50, $p < 0.01$); heart rate increased from 393 at midgestation to 417 at term (F ratio 15.22, $p < 0.01$). These trends in mesor are shown in Fig. 6.

The blood pressure and heart rate trends observed in the pregnant rats can be compared with data obtained from nonpregnant animals. Seven female Sprague-Dawley rats were instrumented and studied in a fashion similar to that used with the pregnant animals. After the rats had recovered from surgery, highly statistically significant circadian rhythms of heart rate were present, with acrophases tightly grouped from 10:40 PM to 1:46 AM. As in the pregnant rats, 24-hour blood pressure excursions were relatively small and were not as consistent as heart rate rhythms. In contrast to findings in the pregnant animals, no long-term trends in mesor (mean 24-hour) values of heart rate or blood pressure were seen in the nonpregnant rats.

Comment

This article represents, to our knowledge, the first description of directly measured blood pressure and heart rate followed longitudinally over the last half of the gestation in the rat. Arterial pressure signals were of high quality, and consistent recordings could be obtained from the eleventh day of gestation until term. The quality and quantity of the data obtained permitted precise analysis of circadian rhythmicity by means of single and group mean cosinor techniques. After the animals had recovered from surgery, the data were examined as 48-hour time epochs, because optimal rhythmic analysis uses epochs of at least double the period of the cycle examined.

Circadian rhythms of heart rate were present in individual animals in 94% of all the epochs studied. These rhythms were reliably present, were statistically significant, were of moderately large amplitude, and showed highly consistent timing of acrophases. In contrast, rhythms of MAP were demonstrable in only 67% of the epochs examined. When present, these rhythms were of low amplitude, and the timing of acrophase was more variable. Although group mean cosinor analysis of heart rate showed consistent and highly statistically significant rhythms, similar analysis of MAP was significant for only two time epochs. The lack of significance for earlier states of gestation was because the timing of the acrophase of rhythms in individual animals was variable and was likely to occur at any place on the 24-hour time span (Fig. 5). Thus peaks of MAP occurring at different times tended to cancel each other out when they were regarded as group data, and population-consistent rhythms could not be demonstrated. The appearance of significant group mean rhythmicity later in gestation occurred because MAP acrophases were synchronized within a closer range. This tighter synchronization of blood pressure rhythms may have been related to advancing gestation or to the possibility that MAP rhythms required a longer postsurgery recovery time when compared with heart rate rhythms to eliminate artifacts caused by surgery and anesthesia.

Previous research with the tail cuff technique has demonstrated a late gestational decline in blood pres-

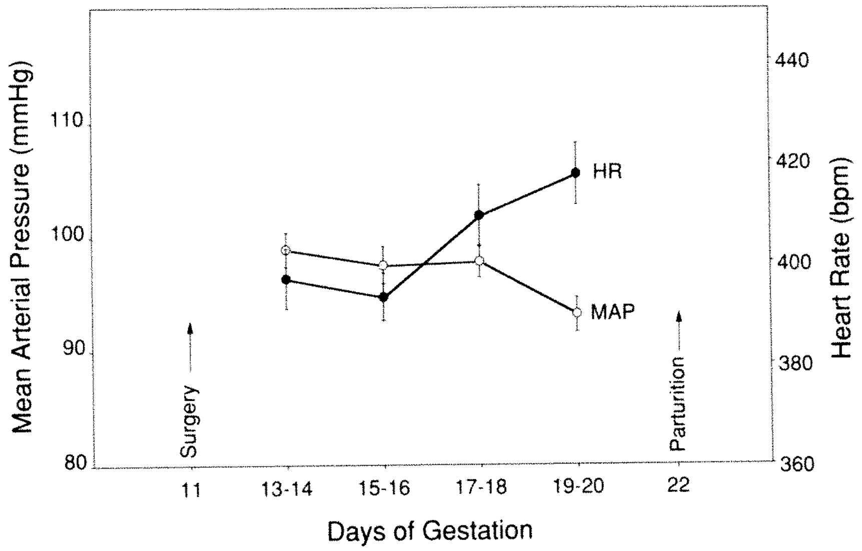

Fig. 6. Cardiovascular function in nine pregnant, normotensive Sprague-Dawley rats. Mean values from all animals for 48-hour epochs of heart rate and MAP over last half of gestation. Mean ± SE is indicated.

Table II. Group mean cosinor analysis of MAP

Days' gestation	*Mesor*		*Amplitude*		*Acrophase*		*n*	*p Value*
	Mean	*95% Confidence interval*	*Mean*	*95% Confidence interval*	*Mean*	*95% Confidence interval*		
14-15	99.8	94.1-105.5	NS	—	NS	—	5	NS
16-17	98.3	93.0-103.6	NS	—	NS	—	6	NS
18-19	97.3	93.4-101.2	1.6	0.72-2.4	4:44 AM	1:13 AM–8:53 AM	7	0.004
20-21	92.2	87.6-96.8	2.0	0.98-3.0	4:09 AM	12:50 AM–8:46 AM	6	0.005

Group mean values and 95% confidence intervals for MAP mesor (mean), amplitude (height of rhythm peak above baseline), and acrophase (timing of peak). Number of animals (out of 9) that exhibited significant rhythmicity of MAP by single cosinor analysis is indicated.

sure in the rat,[8, 18] and studies that use short-term recordings from cannulated rats also have demonstrated that pregnant rats have lower MAP values than control rats 2 to 4 days before term.[9] Our continuous recordings from chronically cannulated rats supplement these previous observations by providing a detailed picture of the time course of the decrease in MAP during rat pregnancy. The decrease begins after the eighteenth day of gestation and continues until term (unpublished observations). During late gestation a pronounced and generalized vasodilation of maternal tissues coincides with final stages of placental maturation. The decrease in MAP can be understood to be a direct consequence of the reduction in total peripheral resistance that accompanies these vascular changes.[19]

The decrease in MAPs occurs in spite of the pronounced increase in cardiac output that has been primarily attributed, in the normotensive animal, to increased stroke volume that results from increased venous return.[11] However, these results clearly demonstrate an increase in heart rate, which is first seen on gestational days 17 to 18 and is maintained thereafter. These observations provide compelling evidence that chronotropic influences also support increased cardiac output in normotensive pregnant rats. Increases in heart rate in late-pregnant rats occasionally have been reported in studies of acute preparations.[15] However, the chronic preparation used, which results in minimal disturbance of the animals, provides important new information about the timing of heart rate increases and their relationship to the decrease in mean arterial pressure.

The reciprocal relationship between MAP and heart rate in late gestation raises the possibility that the baroreceptor reflex plays an important role in maintaining increased heart rate in the late-pregnant rat. This is an intriguing possibility because most previous research has emphasized the role of the baroreceptor reflex in controlling hemodynamic adjustments to phasic postural changes. In the late-pregnant rat increased heart rate is maintained over a 4-day time span (Fig. 6). If the baroreceptor reflex mediates the increased heart rate of late gestation, an explanation must be sought as to why the reflex does not adapt to the sustained decrease in blood pressure that occurs in late pregnancy. The discovery of the inverse relationship between heart rate and MAP in late pregnancy emphasizes the value of the chronic preparation in evaluating the relationship between longitudinal changes in specific hemodynamic systems.

Controversy exists regarding whether the pregnant rat is an appropriate or a useful model to better the understanding of the cardiovascular changes of human pregnancy. The characteristics of murine blood pressure and heart rate observed in this study show both similarities and differences when compared with data obtained from pregnant women. The increase in heart rate observed during murine pregnancy is similar to heart rate changes seen in human pregnancy.[20] Gestational blood pressure monitoring in human beings demonstrates a larger 24-hour amplitude than that seen in rats and has more clearly demonstrable circadian rhythmicity.[2, 3, 21] The recently published study by Cugini et al.[21] shows a decrease in mesor or mean 24-hour blood pressure in clinically healthy pregnant women that occurs in the second trimester and persists in the third trimester, although in the rats observed in this study the decrease in mesor of blood pressure was not seen until late in gestation. We suggest that, although the time of gestation when cardiovascular changes occur may differ between rat and human pregnancy, the direction of the trends is similar and the pregnant rat may be a useful model for studying the cardiovascular consequences of pregnancy.

In summary, we have demonstrated a model for the long-term evaluation of cardiovascular functions in pregnant rats. Highly consistent and statistically significant circadian rhythms were present in heart rate, as were somewhat less consistent rhythms in MAP. This model shows promise for studying regulatory mechanisms of blood pressure and heart rate in normotensive and hypertensive pregnant rodents.

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Bilateral destruction of the fetal paraventricular nuclei prolongs gestation in sheep

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At present the origin of the signal(s) to initiate parturition remains unknown. Experimental studies in sheep have shown that interruption of the fetal hypothalamohypophyseal-adrenal axis will prolong gestation but no studies have shown prolongation of gestation after destruction of fetal neural structures. In this study we placed bilateral lesions of the paraventricular nucleus in five fetal sheep at 118 to 122 days' gestation. Cortiocotropin-releasing factor staining in the median eminence of the hypothalamus was markedly decreased after fetal paraventricular nucleus lesions. Paraventricular nucleus lesions prevented the fetal adrenocorticotropic hormone rise in response to hypotension and the normal predelivery increase in fetal plasma adrenocorticotropic hormone and cortisol and resulted in impaired fetal adrenal growth relative to the rest of the fetus. Lesioned fetuses had not been delivered at 157 days' gestation when they were removed by cesarian section to obtain tissue for histology. Four control fetuses were delivered at 146.5 ± 0.9 days' gestation (mean ± SEM). These findings indicate that a structure in the fetal brain, the fetal hypothalamic paraventricular nucleus, is necessary for parturition to occur. (AM J OBSTET GYNECOL 1991;165:764-70.)

Key words: Paraventricular nuclei, corticotropin-releasing hormone, parturition, adrenocorticotropin, fetal adrenal

The fetal sheep adrenals and pituitary play an indispensable role in the processes that initiate parturition. The classic work of Liggins et al.[1, 2] showed that bilateral adrenal or pituitary ablation delays parturition. Intravascular adrenocorticotropic hormone (ACTH) infusion to the fetus increases fetal adrenal cortisol production and plasma cortisol concentrations, followed by shortened gestation.[3, 4] Infusion of physiologic doses of cortisol into the fetus will also shorten gestation.[5] The preparturient rise in fetal plasma cortisol concentration starts at approximately 127 days' gestation[6] and is preceded by a small rise in fetal plasma ACTH concentration starting at 115 days' gestation.[7] However, to date no studies have clearly shown involvement of fetal neural structures in the fetal mechanisms that initiate parturition.

Corticotropin-releasing hormone, a 41-amino acid peptide that causes release of ACTH both in vivo and in vitro,[8] is present in highest concentration in the median eminence of the hypothalamus.[9] Most of the neurons in the median eminence that contain corticotropin-releasing hormone have their cell bodies located in the parvocellular portion of the paraventricular nucleus of the hypothalamus.[10, 11] Stereotaxically placed electrolytic lesions of the fetal paraventricular nucleus prevent the rise in fetal plasma ACTH concentration that normally accompanies fetal hypotension and hypoxemia.[12] In addition, small glucocorticoid implants placed adjacent to the paraventricular nucleus of fetal sheep not only block the normal ACTH response to hypotension and hypoxemia, but also eliminate immunostaining for corticotropin-releasing hormone in the fetal paraventricular nucleus and median eminence.[13] However, continuous peripheral intravascular infusion of corticotropin-releasing hormone to the sheep fetus does not always result in delivery or produce the normal decrease in maternal progesterone that precedes delivery.[14] In addition, pulsatile intravascular infusion of corticotropin-releasing hormone or arginine vasopressin (a second hormone implicated in ACTH release)[15] or a combination of corticotropin-releasing hormone and arginine vasopressin fail to result in any premature deliveries.[16] However, it is possible that these corticotropin-releasing hormone infusions to the fetus do not mimic normal corticotropin-releasing hormone release patterns.

We hypothesize that the fetal ovine paraventricular nucleus plays a critical role in the delivery of the signals that give rise to parturition. We therefore lesioned the paraventricular nucleus bilaterally in fetal sheep at 118 to 122 days' gestation to determine whether absence of information from these structures would prolong gestation. In both lesioned and sham-lesioned fetuses we measured fetal plasma ACTH and cortisol in the basal

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state, after a hypotensive challenge to the fetus and at frequent intervals to assess any differences in preparturient changes in fetal plasma ACTH and cortisol between paraventricular nucleus lesioned–fetuses and controls.

Material and methods

Care of animals and surgical procedures. Nine Rambouillet-Columbia ewes were mated on a single occasion. To obtain fetal blood samples their fetuses were cannulated with polyvinyl catheters via the carotid artery and jugular vein inserted toward the fetal heart as previously described.[17] All ewes carried a single fetus. Surgery was performed at 118 to 122 days' gestation with coordinates derived from a modification of the stereotaxic atlas of Gluckman and Parsons (1983).[18] Two pairs of overlapping lesions were placed in the paraventricular nucleus of the fetal hypothalamus (lesion group, $n = 5$) (coordinates: AP 18 mm, V 12 mm, L 1 mm; AP 16 mm, V 11, L 1 mm where AP = anteroposterior, V = vertical, and L = lateral) with the use of a Radionics RFG-4A research RF lesion generator system (Kopf Tujunga, Calif.) The tissue temperature at the tip of the electrode was raised to 75° C for 30 seconds. In the sham-lesioned animals (sham group, $n = 4$) the electrode was lowered to the same coordinates without activation of the lesion generator.

All fetuses were allowed to recover for 5 days after surgery before being studied. At the end of the study the ewes and fetuses (lesion group) or newborn lambs (sham group) were anesthetized with halothane. To facilitate perfusion and fixation of the fetal brain the second fetal carotid artery was catheterized with a 14-gauge indwelling catheter pointed toward the brain. The jugular vein on the same side was cut. The fetal head was perfused with one liter of normal saline solution, containing 2.0% sodium nitrite (wt/vol) as a vasodilator, at room temperture, with the perfusion bottle at a height of 1 meter and infusion catheter wide open. Next, the anterior vena cava and anterior aorta were clamped. After this, 1 liter of 0.1 mol/L phosphate-buffered acrolein/paraformaldehyde (Sigma Chemical Co., St. Louis) (2.5/4% wt/vol) was perfused.

After perfusion the skull vault was opened and the brain and pituitary were removed. The hypothalamus was blocked out just anterior to the optic chiasm and caudal to the mammillary bodies, and the pituitary stalk was cut close to the pituitary. The tissue was sunk in several changes of 30% sucrose solution and then sliced at 30 μm on a Reichert freezing microtome. Sections were processed for corticotropin-releasing hormone with the avidin biotin complex method (Elite Kits, Vector Laboratories, Burlingame, Calif.) as previously reported.[19] Anti–corticotropin-releasing hormone was kindly donated by Dr. Wylie Vale of the Salk Institute and was used at a concentration of 1:50,000. The specificity of this antiserum for its respective antigen has been reported elsewhere.[9] Sections were counterstained with methyl green (0.2% wt/vol) and evaluated by light microscopy.

Hormone assays

ACTH. ACTH was measured directly in fetal plasma with kits purchased from INCStar (Stillwater, Minn.) with the following modifications. The assay used INCStar standard serially diluted to the appropriate concentration for the assay (4 through 80 pg · ml^{-1} in zero standard. Samples and control sera were thawed in an ice bath; ice-cold deionized water was used to prepare all INCStar reagents. Samples, antisera, and iodinated ACTH were aliquoted on ice into prechilled 12 × 75 mm borosilicate tubes. Standard, control sera, and sample (50 μl) were aliquoted per assay tube and incubated for 24 hours at 4° C with 50 μl of antisera. Radioiodinated ACTH (50 μl) was then added and further incubated for 24 hours at 4° C. Goat, antirabbit precipitating complex (150 μl) was added to all tubes (excluding total count tubes), and incubated for 20 minutes at room temperature after which 500 μl ice-cold phosphate-buffered saline solution 0.01 mol/L phosphate; 0.3 mol/L sodium chloride; 0.259/L thimerosal was added. The precipiated immune complex was pelleted by centrifugation at 3000 rpm for 30 minutes at 15° C and the supernatant immediately decanted.

Parallelism was shown by serially diluting control sheep and human plasma in zero standard (INCStar). Recoveries were made by the addition of a known quantity of standard to sheep and human control plasma. Interassay coefficients of variation for quality control samples of fetal plasma containing 28.7 ± 2.99 pg · ml^{-1} ACTH was 10.4% ($n = 10$). Intraassay coefficient of variation for qualtiy control sample containing 29.05 ± 1.76 pg · ml^{-1} ACTH ($n = 5$) was 6.1%. The limit of detection of this assay (i.e., 90% binding of radiolabeled ACTH in the assay tube) compared with the binding of radiolabeled ACTH in the absence of added ACTH (B/Bo) was 5.0 pg · ml^{-1}.

Cortisol[6] and progesterone[20] were measured by radioimmunoassay as previously described.

Blood samples

Daily samples. Fetal and maternal blood samples were taken once daily between noon and 3 PM. Blood sampling was started on day 5 after surgery and continued until term (sham group) or necropsy (lesion group). A total of 5 ml of fetal blood was taken from the fetal carotid artery at each sampling time. Blood samples were drawn into cold disposable plastic syringes, immediately chilled in ice, transferred to chilled heparinized 13 × 100 polypropylene tubes, and centrifuged at 4° C at 2000 rpm for 10 minutes. The supernatant

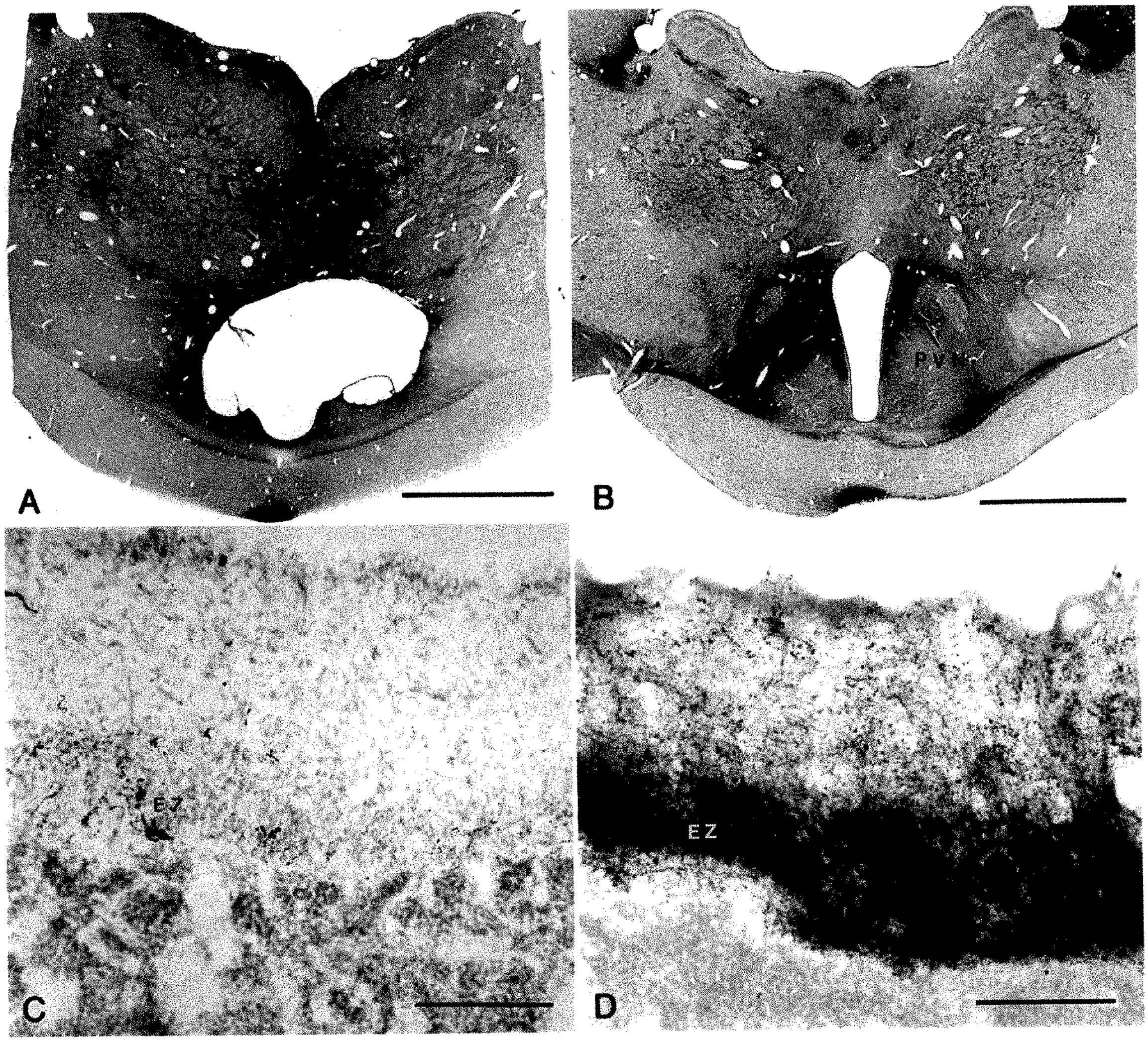

Fig. 1. Fetal hypothalamic sections stained for corticotropin-releasing hormone *(CRH)* **A,** Coronal section of brain in fetal sheep with bilateral lesions (lesion group) of the fetal hypothalamic paraventricular nucleus *(PVN)*. **B,** Coronal section of brain in fetal sheep with sham lesions (sham group) of PVN. **C,** Coronal section of median eminence *(ME)* of fetal sheep, lesion group, showing greatly reduced staining for corticotropin-releasing hormone in the median eminence external zone *(EZ)*. **D,** Coronal section of median eminence of sham group showing intense corticotropin-releasing hormone staining in the external zone. *Bars* indicate 3.75 mm (**A** and **B**) and 140 μm (**C** and **D**).

plasma was transferred to plastic vials, immediately flash frozen in liquid nitrogen, and stored at −20° C until assayed. After each sample, the fetal red blood cells were aseptically returned to the fetus.[21]

Fetal response to hypotension. Five days after surgery fetuses were subjected to a hypotensive challenge. Fetal mean arterial blood pressure was monitored with a Statham pressure transducer (P23ID) and recorded on a Grass polygraph (model 7). Heparinized fetal blood samples (5.0 ml per sample) were taken at −15, −5, +10, +20, and +30 minutes and monitored for blood gas, pH, and hematocrit values. Plasma was removed, frozen in liquid nitrogen, and stored at −20° C until assayed. Fetal blood pressure was reduced by 50% of baseline value by infusing nitroprusside in saline solution to the fetal jugular vein (starting at time = 0, at a rate of 100 $\mu g \cdot min^{-1}$ and adjusting the flow to effect). at +10 minutes the nitroprusside infusion was stopped and fetal blood pressure was allowed to recover.[12]

Blood gas values. Blood gas and pH values were measured on an ABLII blood gas analyzer (Radiometer, Copenhagen).

Statistical analysis

Fetal response to hypotension. The fetal plasma ACTH concentrations were averaged over both the prenitroprusside and postnitroprusside infusion periods to yield two independent data points for each animal. The average values for treated and control animals were then analyzed with the paired Student *t* test to assess increment within group and the nonpaired Student *t* test to compare the lesion group with the sham group.

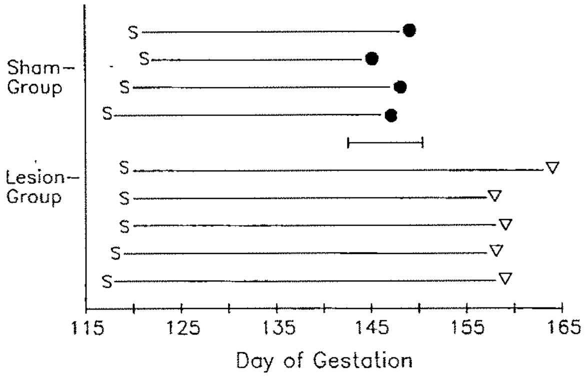

Fig. 2. Outcomes of individual preparations: Gestational ages at surgery *(S)* and spontaneous delivery (●) in sham lesioned group (sham group) or elected necropsy (∇) of fetal sheep with bilateral lesions of the hypothalamic paraventricular nucleus (lesion group). Age at spontaneous delivery of sham group was 146 ± 0.9 (x ± SEM); ├┤ represents 95% confidence limits for spontaneous delivery in sham group.

The Bonferroni correction was used for multiple comparisons.

Daily hormone concentrations. Average hormone concentrations for ACTH, cortisol, and progesterone on the last day of gestation in the sham-lesioned group are compared with the concentrations at term +10 days' gestation for the lesion group with the nonpaired Student *t* test with α set at 0.05.

Prolongation of gestation. Necropsy was performed at ≥157 days' gestation in the lesioned fetuses. We made the initial rationale that the controls in this study would behave similarly to our previous controls and be delivered at about 147.1 ± 5.27 days' gestation (mean ± SD; $n = 7$). We determined that if five animals went through the experimental protocol and had not been delivered by 157 days' gestation the probability for a one-sided test would be as follows:

$$\Pr(\bar{x} > 147) = \Pr\left(\frac{\bar{x} - \mu}{S/\sqrt{n}} > t\epsilon\alpha\right)$$
$$= \frac{147 - 157}{5.27\sqrt{5}} = -4.24$$

or $p < 0.01$. Because the days' gestation at necropsy were elected events and are therefore censored data, statistical comparisons are nonsensical.

Data throughout are presented as mean ± SEM except where noted otherwise.

Results

Fetal pH, P_{CO_2}, and P_{O_2} of the lesion group and sham group (7.38 ± 0.004 vs 7.37 ± 0.001, 50.9 ± 0.8 vs 51.1 ± 0.7 mm Hg, and 21.3 ± 0.4 vs 19.8 ± 0.8 mm Hg) averaged over the course of the experiment were not different.

Coronal sections through the brain and hypothalamus of the paraventricular nucleus—lesioned fetuses

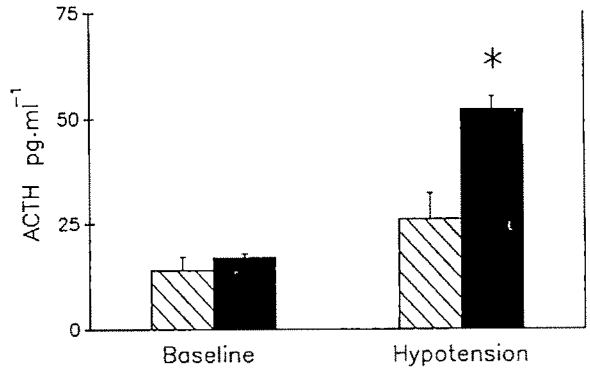

Fig. 3. Fetal plasma ACTH (mean ± SEM) in ovine fetuses with bilateral lesion of the hypothalamic paraventricular nucleus (▨) or sham lesion (■) in response to 50% drop in arterial blood pressure induced by nitroprusside infused intravascularly to fetus from 0 to 10 minutes at approximately 125 days' gestation. Baseline ACTH is average of two samples (−15 and −5 minutes). ACTH during hypotension is average of three samples (+10, +20, and +30 minutes after the commencement of hypotension). *$p < 0.05$ When lesion and sham groups are compared at same time period and compared with baseline in same group.

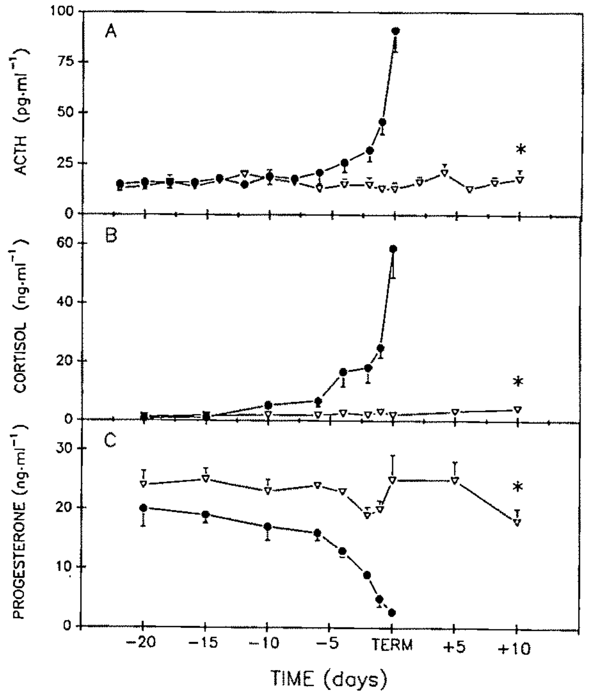

Fig. 4, A, Plasma ACTH concentrations of fetal sheep with bilateral lesions of the hypothalamic paraventricular nucleus (lesion group, ∇) or sham lesion (sham group, ●) from term −22 days' gestation to term +10 days' gestation (term, 146.5 ± 0.9 days' gestation, mean ± SEM in sham group). *$p < 0.05$ When compared with sham group at term. **B,** Plasma cortisol concentrations of lesion group (∇) or sham group (●) from term −20 days' gestation to term +10 days' gestation. *$p < 0.05$ When compared with sham group at term. **C,** Plasma progesterone concentrations of ewes bearing fetuses of lesion group or (∇) sham group (●) from term −20 days' gestation to term +10 days' gestation. *$p < 0.05$ When compared with ewes bearing sham group at term.

Table I. Body weight, combined adrenal weight, and organ weights of fetal sheep with bilateral lesions of the hypothalamic paraventricular nucleus or sham lesion

	Body weight (kg)	*Adrenal weight (mg)*	*Adrenals (% BW)*	*Heart (% BW)*	*Lungs (% BW)*	*Liver (% BW)*
Lesion group	5.26 ± 0.6*	487 ± 69.6	0.0096 ± 0.0014*	0.06 ± 0.07	3.2 ± 0.5*	2.1 ± 0.2
Sham group	3.5 ± 0.5	755 ± 123.6	0.0213 ± 0.0029	0.06 ± 0.03	1.8 ± 0.2	2.3 ± 0.3

BW, Body weight. Cesarean section of lesion group performed at ≥157 days' gestation; term of sham group = 146.5 ± 0.9 days' gestation.

*$p < 0.05$ When compared with sham group at term. Average absolute weight of adrenals of lesion group was not less than that of sham group but $p = 0.07$ when compared. Values are mean ± SEM.

(lesion group) and the sham-lesioned fetus (sham group) are presented in Figs. 1, *A* and 1, *B*, respectively. The lesions created were approximately 3 mm × 3 mm × 2 mm in diameter in the anteroposterior, vertical, and lateral directions, respectively. Lesions produced in all five fetuses were similar in size and location. When studied with light microscopy there was no consistent damage to other nuclei; the suprachiasmatic nucleus was damaged in one lesioned fetus but not in the other four. The anterior columns of the fornix were damaged bilaterally in all lesioned fetuses. Bilateral damage to the wall of the ventricle was inevitable in the lesion group. Generally, the lesion extended from the anterior aspect of the optic chiasm to the level of the median eminence. Immunocytochemistry revealed that there were a few scattered corticotropin-releasing hormone–positive cells left on the periphery of the lesion in two fetuses. However, the lesion-group had greatly reduced corticotropin-releasing hormone–staining in the external zone of the median eminence when compared with the sham-group fetuses (Fig. 1, *C*, and *D*).

The data on length of gestation are presented in Fig. 2. The fetuses of the lesion group were harvested at ≥157 days' gestation as shown. Average length of gestation for the sham group was 146.5 ± 0.9 days (mean ± SEM).

Fig. 3 shows the fetal plasma ACTH response to a 50% drop in mean arterial blood pressure produced by nitroprusside infusion at 5 days after surgery. Prehypotension baseline ACTH values were not different in the lesion group (14 ± 3.2 $pg \cdot ml^{-1}$) and sham group (17 ± 0.9). After hypotension the average fetal plasma ACTH concentration in the lesion group was 26 ± 6.1 $pg \cdot ml^{-1}$ and was not different from baseline. In contrast the sham group had average ACTH concentrations after nitroprusside hypotension that were greater than baseline (52 ± 3.2 vs 17 ± 0.9 $pg \cdot ml^{-1}$) ($p < 0.05$) and greater when compared with the lesion group after hypotension ($p < 0.05$).

Daily fetal plasma ACTH concentrations over the course of the experiment (Fig. 4) were all greater than the lower limit of sensitivity of the assay (5.0 $pg \cdot ml^{-1}$). The ACTH profile of the lesion group appears quite linear over the course of the experiment whereas that of the sham group shows the steep preparturient rise typical of intact fetuses. Ten days after term the lesion group still had less fetal plasma ACTH (18 ± 4.7 $pg \cdot ml^{-1}$) than the sham group at term (91 ± 10.4 $pg \cdot ml^{-1}$) ($p < 0.05$). The fetal plasma cortisol concentrations of the lesion group (Fig. 4, *B*) appear linear over the course of the experiment when compared with the sham group. Ten days after term the lesion group still had less ($p < 0.05$) fetal plasma cortisol (4.2 ± 1.8 $ng \cdot ml^{-1}$) than that of the sham group (58.8 ± 9.9 $ng \cdot ml^{-1}$). Even at 10 days after term the maternal plasma progesterone concentrations (Fig. 4, *C*) of the lesion group are typical of ewes before parturition and are greater ($p < 0.05$) than those of sham group at term (17 ± 1.2 vs 3 ± 0.8 $ng \cdot ml^{-1}$).

The data on body and organ weights are shown in Table I. The lesion group had larger body weights ($p < 0.05$) than those of the sham group (5.3 ± 0.6 vs 3.5 ± 0.5 kg). When expressed as percentage of body weight, adrenals of the lesion group were smaller ($p < 0.05$) than those of the sham group (0.0096% ± 0.0014% vs 0.0213% ± 0.0029%), lungs of the lesion group were larger ($p < 0.05$) than those of the sham group (3.2% ± 0.5% vs 1.8% ± 0.2%), and heart and liver were not different between the lesion group and sham group (0.6% ± 0.07% vs 0.6% ± 0.03%, and 2.1% ± 0.2% vs 2.3% ± 0.3%, respectively). In absolute terms, the adrenals of the lesion group were not different from those of the sham group (487 ± 69.6 vs 755 ± 123.6 mg, respectively) but the p value was 0.07.

Comment

The paraventricular nucleus was destroyed bilaterally in all lesion-group fetuses. The completeness of the bilateral paraventricular nucleus lesion was shown by the lack of corticotropin-releasing hormone–staining neurons in the normal location of the paraventricular nucleus (except a few scattered cells in two fetuses). In addition, the lesion group had greatly reduced corticotropin-releasing hormone in the external zone of the median eminence as determined by immunocytochemistry when compared with the sham group. At necropsy the lesion group had exceeded the average age of the sham group at term by 5 SDs and showed no signs of delivery.

Fetal plasma concentrations of both ACTH and cortisol of the lesion group at the time of necropsy were similar to those of the sham-lesion group early in the study and never showed the well-documented preparturient rise observed in normal fetuses in either ACTH[22] or corisol[6] and that occurred in the sham-lesion group (Fig. 4, *A*,and *B*). Likewise, by the time of necropsy maternal progesterone concentrations of the lesion group failed to exhibit the normal reduction that was seen in the sham group (Fig. 4, *C*) at term.

Fetal sheep with pituitary lesions[23] exhibit hypoplastic adrenals and prolongation of pregnancy without an increase in cortisol value above baseline. Antolovich et al.[24] were able to prolong gestation by disconnecting the pituitary from the hypothalamus (HPD) in fetal sheep while preserving the blood supply to the pituitary. Contrary to our findings in paraventricular nucleus–lesioned fetuses, HPD fetuses had baseline ACTH concentrations in plasma that were more than twice as great as the sham-HPD group at 133 to 138 days' gestation (127.5 ± 3.8 vs 60.2 ± 10.8 $\mathrm{pg \cdot ml^{-1}}$) and highest in postmaturity (145.2 ± 11.7 $\mathrm{pg \cdot ml^{-1}}$) without accompanying rises in cortisol. The discrepancy between the changes in fetal plasma ACTH and cortisol is hard to explain and suggests a difference in the responsiveness of the adrenals of the HPD fetuses to ACTH when compared with the sham-group fetuses.

It could be argued that the damage we observed to the fornix prevented the lesion group from delivery at term. However, it is well documented that lesions of the fornix in adult rats cause a hypersecretion of ACTH secretagogues into the portal system,[25] which, if anything, would have been expected to hasten delivery. It therefore seems logical to argue that prolongation of gestation was a result of loss of the paraventricular nucleus, a major contributor to the secretion of hormones into the portal system, rather than damage of the fornix. In any event our data show that destruction of the fetal neural structures in this area prolongs gestation.

The paraventricular nucleus is a large nucleus that has been shown to contain more than 30 neurotransmitters. If, as our results strongly suggest, the fetal paraventricular nucleus is a central participant in initiation of labor, what factor or factors contained therein are involved? Also, what pathways provide information on fetal maturity to the paraventricular nucleus or does it act as an anautonomous biological clock? In adults the corticotropin-releasing activity of the paraventricular nucleus via the median eminence is greatly influenced by inputs from the nucleus of the solitary tract, the amygdala and the hippocampus.[25-27] We hypothesize that a similar situation exists in fetal sheep, allowing the fetal brain to sum a multitude of sensory inputs in determining maturational status of the fetus to initiate parturition when a critical level of maturity has been achieved. Obviously further studies are needed to answer these interesting and challenging questions.

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The effect of interleukin-1 on adhesion formation in the rat

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The potential role of interleukin-1 in postoperative adhesion formation was examined. Cecal abrasion gave a consistently higher adhesion score when compared with sham laparotomy, on the basis of adhesion number, density, and vascularity, and so was chosen for use in further studies. The extent of serosal bleeding during cecal abrasion did not affect adhesion scores. Intraperitoneal injection of 10 μg murine recombinant interleukin-1α in cecally abraded animals on the day of surgery and on the following 4 days resulted in a significant increase in adhesion scores when compared with those of cecally abraded animals injected with vehicle alone. Adhesions enhanced with murine recombinant interleukin-1α, which were thicker and more vascular, were equivalently enhanced at doses from 10 to 10,000 ng, implying maximal response over that range. Rats not operated on and receiving recombinant interleukin-1α 2 weeks after injury had increased adhesion formation. These results demonstrate that interleukin-1α may be an important short-term mediator of postsurgical adhesion formation. (AM J OBSTET GYNECOL 1991;165: 771-4.)

Key words: Adhesion, interleukin-1, cecum

Pelvic adhesions, which may result from a surgical procedure involving the parietal and visceral peritoneum or a primary inflammatory or infectious process, are of major concern to the reproductive surgeon because they can contribute to infertility. An understanding of the pathogenetic mechanisms of adhesion formation is important for studies aimed at developing strategies for their prevention.

One proposed mechanism for the formation of permanent fibrous adhesions is the inability of the injured peritoneum to remove deposited fibrin.[1] This may result from impaired production of plasminogen activators, which are responsible for the formation of plasmin and subsequent fibrinolysis. Such activity may be depressed with peritoneal injury.[1] DiZerega[2] has reviewed the pathologic features and pathogenesis of peritoneal healing. In the early period after injury (up to 3 days), the most pronounced elements in the healing lesion are polymorphonuclear cells and fibrin. Later there is an influx of new elements, including macrophages, mesothelial cells, and the development of angiogenesis. These infiltrating cells may release cytokines and other growth factors that may play a role in the adhesion process.

A few existing studies have led us to believe that cytokines such as interleukin-1 (IL-1) may be involved in both the normal healing response and the abnormal response to peritoneal injury that leads to postoperative adhesion formation. IL-1 is a cytokine produced by the host during the inflammatory response after microbial invasion or physical injury.[3] Multiple in vivo effects of IL-1 have been reported, including vascular endothelial inflammatory changes leading to expression of adhesion proteins, regulation of fibrin deposition and lysis, increase in collagenase production of fibroblasts, collagen type I and IV deposition by fibroblasts and epithelial cells, stimulation of fibroblast proliferation, and modulation of hematopoiesis and granulopoiesis.[3] The availability of recombinant murine IL-1[4] has allowed us to directly examine the potential role of IL-1 in peritoneal healing.

Material and methods

Rats. Female Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing between 200 and 300 gm were maintained in accord with good husbandry standards and fed at will.

Surgical procedures. All procedures were performed with animals under general anesthesia with pentobarbital sodium (A.J. Buck and Son, Cockeysville, Md.), 39 mg/kg. Each animal was weighed, shaved, and earmarked. A midline abdominal incision was then made, first through the skin, then through the peritoneum. The cecum was identified and brought

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Table I. Adhesion scoring system

	Adhesion score
Extent	
None	0
Confined to traumatized area	1
Confined to nontraumatized area	1
In traumatized and nontraumatized areas	2
Type	
Filmy	1
Opaque, nonvascular	2
Opaque, vascular	3
Dense	4

through the incision. The distal 2 cm of the anterior cecum was scraped 15 times with a scalpel, rendering the serosal surface disrupted and hemorrhagic. The cecum was then dropped back into the abdomen, the peritoneum was closed by a continuous suture of 4-0 nylon, and the skin was closed with staples. In the sham laparotomy group, the skin and peritoneum were opened and then closed as described earlier. Surgery was tolerated well with no sequelae except for occasional incisional hernias. Weights were recorded in all experiments before procedures and before death, but no consistent pattern could be identified. Initially, rats were killed 2 weeks after surgery. Since we have found adhesions to be well formed within 1 week after surgery, subsequent scoring was done at a 7-day interval from surgery.

Adhesion scoring. Adhesions were scored by a blinded observer using criteria relating to extent and type, as shown in Table I. The total score in each animal represents a composite of individual scores given to each adhesion.

Recombinant IL-1α. Murine recombinant IL-1α complementary deoxyribonucleic acid was expressed in *Escherichia coli.*[4] The biologic activity of the recombinant IL-1α was verified in the lymphocyte-activating factor assay by binding to the IL-1 receptor on EL4 6.1 cells, by induction of colony-stimulating factor and cartilage resorption in vitro, and by induction of serum amyloid P, cerebrospinal fluid, IL-6, and fever in vivo. Endotoxin was less than one part in 100,000 by the Limulus assay. Phosphate-buffered saline solution was used as vehicle and placebo.

Statistics. Statistical analysis was performed by the Kruskal-Wallis nonparametric test or paired t test when appropriate.

Experiments

Cecal abrasion. Cecal abrasion was examined for suitability as a model of adhesion formation. Body weight was measured and recorded before surgery and again before death. Necropsy was performed 2 weeks after operation, and adhesions were recorded.

The effect of bleeding. Cecal abrasion can result in petechial hemorrhage at the serosal surface and occasionally in frank bleeding. To examine the effect of bleeding as a variable in the model that could alter the results in the cecal abrasion model, we selected a group of rats with more extensive bleeding from the vessels on the serosal surface of the cecum and compared them with the more typical cecal abrasion, i.e., a hemorrhagic surface but not bleeding.

Potentiation of adhesions by recombinant IL-1α. In this phase of the studies recombinant IL-1α was given intraperitoneally at a dose of 10 μg per rat diluted in phosphate-buffered saline solution to a total volume of 1 ml. This dose was chosen on the basis of previous work using continuous-infusion pumps of recombinant IL-1α.[5, 6] Injections were given daily for 5 days, starting on the day of surgery. Four groups of animals were studied: cecal abrasion with recombinant IL-1α, cecal abrasion with vehicle, no surgery and recombinant IL-1α, and no surgery and vehicle. In our initial experiments, necropsy was performed 2 weeks later and adhesions were scored. However, in later kinetic experiments we found that adhesion scoring did not change between 7 and 14 days after operation; therefore all subsequent necropsies were performed 7 days after cecal abrasion.

Dose response. Five daily intraperitoneal injections of recombinant IL-1α were given at four different doses (0.01, 0.1, 1, and 10 μg per rat, each in a total volume of 1 ml of phosphate-buffered saline solution) to cecally abraded animals. Vehicle was administered to a cecal abrasion control group. Animals were studied 1 week after laparotomy, and adhesions were scored.

Acute versus delayed effect of recombinant IL-1α. The effect of time of administration of recombinant IL-1α after surgery on adhesion formation was studied. Daily injections of 10 μg of recombinant IL-1α for 5 days were given to one group starting on the day of surgery and to a second group 2 weeks after surgery. Each recombinant IL-1α–treated group had a comparable vehicle-treated control group set up. Animals were killed 1 week after recombinant IL-1α injections were initiated, and adhesions were scored.

Results

Cecal abrasion. Adhesion scores were significantly and consistently higher in the cecal abrasion group than in the sham laparotomy group (mean ± SEM, 6.2 ± 0.6 vs 1.1 ± 0.1, respectively; $p < 0.05$). Adhesions resulting from cecal abrasion were typically between the cecum and the uterine horn, and often there was a second adhesion from the cecum to the small intestine or the other uterine horn. Because of the consistency of adhesion formation, this procedure was chosen for subsequent experiments.

Effects of bleeding. Adhesion scores did not differ in the two groups between animals with significant bleeding from the serosal surface and animals not

Table II. Effect of IL-1 on adhesion formation

Surgical procedure	*Treatment*	*No.*	*Adhesion score (mean ± SEM)*
Cecal abrasion	IL-1	6	14.3 ± 1.7*
Cecal abrasion	Vehicle	5	7.6 ± 0.9
No surgery	IL-1	5	0
No surgery	Vehicle	4	0

Treatment, Recombinant IL-1α was given at a dosage of 10 μg per rat intraperitoneally in 1 ml phosphate-buffered saline solution from the day of surgery for five daily treatments. Vehicle control received 1 ml phosphate-buffered saline solution. Scoring according to Table I 7 days after surgery.

*$p < 0.05$.

Table III. Effect of IL-1 dose on adhesion score after cecal abrasion

Dose of recombinant IL-1α (μg)	*No.*	*Adhesion score (mean ± SEM)*
0	6	3.0 ± 0.7
0.01	6	6.6 ± 1.1*
0.1	6	6.3 ± 1.3*
1	6	4.5 ± 0.8*
10	6	9.3 ± 1.4*

*$p < 0.05$ for vehicle-treated control. Scoring according to Table I 7 days after surgery.

Table IV. Adhesion development after cecal abrasion and short-term (days 0 through 4) versus delayed (days 14 through 19) administration of IL-1

Dosing schedule	*No.*	*Adhesion score (mean ± SEM)*
Days 0-4, recombinant IL-1α	6	14.0 ± 1.5*
Days 0-4, vehicle	6	6.5 ± 1.5
Days 14-19, recombinant IL-1α	6	3.2 ± 1.2
Days 14-19, vehicle	5	2.4 ± 0.7

*$p < 0.05$.

bleeding from the cecal abrasion (mean ± SEM, of 3.0 ± 1 vs 3.0 ± 0.7, respectively). This suggests that bleeding and fibrin do not increase adhesion formation. Therefore all animals, independent of the degree of hemorrhage, were used in all subsequent studies.

Potentiation of adhesions by recombinant IL-1α (Table II). With the nonparametric Kruskal-Wallis test, the four study groups were found to be different ($p = 0.00005$). Recombinant IL-1α given to cecally abraded animals resulted in significantly higher adhesion scores than those found in cecally abraded animals treated with vehicle. Adhesions associated with recombinant IL-1α injections were typically more extensive and more vascular. Adhesions were absent in animals undergoing only laparotomy, whether treated with recombinant IL-1α or vehicle alone.

Dose response (Table III). The Kruskal-Wallis test showed a difference between all groups ($p = 0.0244$), but when the control was removed and the same test repeated, groups given different recombinant IL-1α doses were then not different ($p = 0.09$). Adhesion scores were significantly higher in each group when compared with vehicle. Essentially the same potentiation of adhesion formation was obtained from 0.01 to 10 μg of recombinant IL-1α.

Short-term versus delayed effect of recombinant IL-1α (Table IV). The Kruskal-Wallis test showed a difference between the groups ($p = 0.0019$). Adhesion scores were significantly higher when cecally-abraded animals were given intraperitoneal recombinant IL-1α injections for 5 days beginning the day of surgery and for the next 4 days, then given vehicle alone, as previously shown. No adhesions were found when recombinant IL-1α was given for 5 days beginning 2 weeks after laparotomy and cecal abrasion.

Comment

Few studies have addressed the role of IL-1 in the normal and abnormal peritoneal healing response. Human recombinant IL-1 was found to enhance radiation-induced adhesions and death in mice.[7] In that study the timing of administration of IL-1 in relationship to the injury causing adhesions, i.e., radiation, was found to be critical. IL-1 enhanced adhesions when it was given daily from 3 days before radiation, including 1 day after; IL-1 did not enhance adhesions when given over the 4-day period after abdominal radiation. As the mechanism of radiation-induced injury may differ substantially from that of surgical injury, we thought it appropriate to study the effect of IL-1 directly after surgical trauma. In previous studies from this laboratory[5, 6] we examined the effects of IL-1 administered by continuous infusion with implanted osmotic pumps and observed that the pumps containing IL-1, but not those containing saline solution, became engulfed in granulation tissue that later contracted to a tough fibrous capsule. These results suggested to us that IL-1 might contribute to adhesion formation, and we therefore undertook this study.

IL-1 is synthesized and released in response to injury.[3] Thus, if it were to play a physiologic role in the healing process, it must be secreted in the period during and immediately after surgery. In addition, sepsis during surgery, particularly with gram-negative organisms, would be expected to augment IL-1 production.[8] We therefore elected to treat animals with IL-1 beginning with the completion of surgery. The intraperitoneal route was chosen to mimic the local production of IL-1. Daily intraperitoneal injection of IL-1 for 5 days, starting on the day of peritoneal injury, consistently resulted in increased adhesion scores 7 days after injury. In our pilot studies we observed that IL-1 causes (1) more adhesions and (2) more vascular adhesions; therefore a scoring system was developed so that each of these could be reflected separately in quantifying the IL-1 effect. No direct dose-response relationship was shown, since adhesion scores were similar from the lowest test dose of IL-1 (0.01 μg) to the highest test dose (10 μg) given, suggesting a maximal response over the tested dose range. Delayed administration of IL-1 2 weeks after injury did not increase adhesion scores above control level, thus suggesting that IL-1 enhanced adhesion formation only in the acute stage of peritoneal injury. When IL-1 was administered for 5 days to nontraumatized animals, it did not result in adhesions.

IL-1 could contribute at several different points to peritoneal adhesion formation. We have previously shown that IL-1 may lead directly to granuloma formation when given by continuous release by minipump[5] or as a slow-release encapsulated polymer.[9] However, with injection once daily it appears only to potentiate rather than to induce adhesion formation, suggesting that it acts to amplify the events occurring normally with surgical injury. IL-1 induces through a cyclic adenosine 5′-monophosphate–dependent mechanism the synthesis of both plasminogen activators[10] and inhibitors of plasminogen activity.[11, 12] In addition, IL-1 has been shown to induce the expression of adhesion proteins in endothelial cells.[13, 14] Moreover, IL-1 is a potent inducer of IL-8, an important chemotactic factor.[15] Since the extent of bleeding did not alter the adhesion scores, abnormal fibrinolysis may not be the entire pathogenetic mechanism responsible for adhesion formation in this model. We suggest as an alternative hypothesis that adhesion molecules that are not normally expressed in abdominal and pelvic structures are up regulated by IL-1. These adhesion molecules provide the substratum on which cell-cell adherence is enhanced and from which adhesions will ultimately form.[16]

As we have shown IL-1 to enhance the formation of adhesions, the question of whether endogenous IL-1 participates in the adhesion formation process becomes important. Since IL-1 is secreted by macrophages and other cells known to participate in the peritoneal response to injury, it seems reasonable to assume that IL-1 may be present and active in such circumstances. The amount of IL-1 liberated may be related to the extent of the inflammatory process either present in the peritoneal cavity at the time of surgery or directly induced by the trauma associated with surgery. Pelvic infection would be expected to further augment IL-1 production. Thus down regulation of interleukin may have therapeutic implications in the prevention of adhesion formation after gynecologic surgery.

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Acute fetal ductal occlusion in lambs

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To assess fetal right ventricular and hemodynamic responses to acute occlusion of the ductus arteriosus, the pulmonary pressure, right and left ventricular output, and right ventricular dimensions of five fetal lambs were measured with simultaneous echocardiographic monitoring. Ductal occlusion resulted in a rise in pulmonary arterial pressure, a decrease in right ventricular output of 68%, an increase in left ventricular output of 18%, and a fall in combined cardiac output of 34%. The right ventricular systolic dimension increased, and the shortening fraction decreased from 0.41 to 0.14. Tricuspid regurgitation started within two heartbeats after ductal occlusion and resolved as soon as the occlusion was released. Acute fetal ductal occlusion imposes a marked increase in the right ventricular afterload, resulting in reversible triscuspid regurgitation. (Am J Obstet Gynecol 1991;165:775-8.)

Key words: Fetal ductus arteriosus, fetal echocardiography, cardiovascular hemodynamics

Fetal ductal constriction and triscupid valve regurgitation may occur in patients who have been treated with indomethacin for premature labor.[1, 2] It has been shown in animal experiments that chronic fetal ductal constriction-occlusion can cause morphologic pulmonary vascular changes from fetal pulmonary hypertension, resulting in persistent pulmonary hypertension of the newborn.[3-5] Degenerative myocardial changes in the right ventricular free wall and in the papillary muscles of the tricuspid valve after chronic ductal constriction were studied to determine the cause of tricuspid regurgitation.[6] There is, however, little known about the hemodynamic effects of acute fetal ductal constriction-occlusion of the right ventricle. To study acute changes in fetal right ventricular afterload, an animal model of acute fetal ductal occlusion was developed.

Material and methods

All animal experiments conformed to the guiding principles of the American Physiological Society. Five pregnant ewes (120 to 130 days' gestation; term is 148 days) were anesthetized with pentobarbital. The uterus was exposed by cesarean section; after the fetal chest and left forelimb were exposed through a uterine incision, fetal thoracotomy was performed in the third intercostal space. An electronic pressure catheter (Millar Instruments, Inc., Houston) was placed in the main pulmonary artery, and ultrasonographic flowmeters were placed around the main pulmonary artery and ascending aorta. Ultrasonographic measurements

Table I. Change of parameters from baseline during acute fetal ductal occlusion

	Baseline		*Ductal occlusion*		*Paired Student's t test results*
	Mean	*Range*	*Mean*	*Range*	
Systolic PAP (mm Hg)	57	46-67	94	79-102	$p \leq 0.0005$
Diastolic PAP (mm Hg)	45	40-50	56	52-61	$p \leq 0.01$
RVEDD (cm)	4.1	3.2-5.1	5.9	5.1-6.6	NS
RVESD (cm)	2.4	1.8-3.1	4.9	4.5-5.6	$p \leq 0.005$
RV SF (%)	0.41	0.38-0.43	0.14	0.25-0.07	$p \leq 0.025$
Q PA/Q Ao	1.7	1.4-1.9	0.45	0.39-0.51	$p \leq 0.0005$

PAP, Pulmonary artery pressure; *NS,* not significant; *RVEDD,* right ventricular end-diastolic pressure; *RVESD,* right ventricular end-systolic pressure; *RV SF,* right ventricular shortening fraction (RVEDD − RVESD/RVEDD); *Q PA/Q Ao,* aortic flow/pulmonary artery flow.

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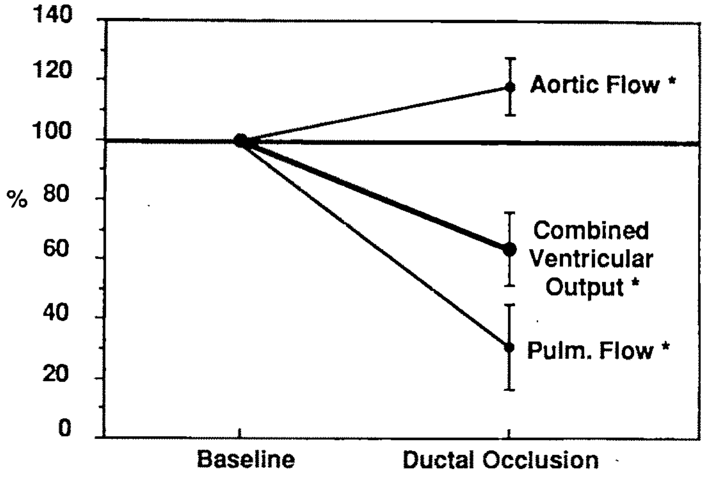

Fig. 1. Pulmonary flow, aortic flow, and total cardiac output changes during mechanical occlusion of fetal ductus arteriosus. Values are given as percentage change from baseline (mean ± SD).

(right ventricular end-diastolic and end-systolic dimension, tricuspid regurgitation) were performed (CFM 700, VingMed, Horten, Norway) with a 5 MHz sector scanner and 4 MHz continuous-wave Doppler echocardiography. We used color Doppler equipment to detect tricuspid valve regurgitation; continuous-wave Doppler echocardiography confirmed the holosystolic and high-velocity nature of the jets. The methods of DeVore et al.[7] were used to measure right ventricular shortening from the four-chamber view with M-mode echocardiography. The pulmonary artery pressure tracing was displayed on the ultrasonographic screen. Acute fetal ductal occlusion was mechanically induced for 30 seconds with a tightened rubber band placed around the ductus arteriosus. During multiple episodes of ductal ligature tightening, occlusion of the ductal flow was confirmed with continuous-wave Doppler echocardiography taken from the right ventricular outflow tract, as previously described.[8]

Each fetus served as its own control. Parameters recorded at baseline and during ductal occlusion were compared by using the paired Student *t* test. A p value of <0.05 was considered to be significant.

Results

During acute ductal occlusion there was a significant increase in pulmonary artery systolic and diastolic pressures from baseline level and a significant decrease in the ratio of pulmonary artery flow to aortic flow (Table I). Right ventricular end-diastolic and end-systolic dimensions increased gradually between 2 and 20 seconds after ductal occlusion with a substantial simultaneous decrease in right ventricular shortening fraction. The right ventricular diameter was unchanged at the onset of tricuspid regurgitation. Pulmonary flow decreased 68% (range 57% to 72%) from baseline, and aortic flow increased 18% (range 15% to 24%). Combined ventricular output fell 34% (range 24% to 55%; Fig. 1). Tricuspid regurgitation developed consistently within two heartbeats after the onset of ductal occlusion in all cases and disappeared immediately after the occlusion was released (Fig. 2). All physiologic parameters returned to baseline levels after the ductus arteriosus was reopened.

Comment

This animal model demonstrated that tricuspid regurgitation occurred immediately after an increase in right ventricular afterload. Main pulmonary artery flow decreased as pulmonary artery pressure rose. This was consistent with an inverse relationship between the afterload and stroke volume of a ventricle. There was also a significant increase in left ventricular output, probably because of right-to-left shunting across the foramen ovale and increased pulmonary arteriolar flow. This flow redistribution maintains peripheral organ perfusion and may explain the clinical experience that even severe ductal constriction is usually well tolerated for 2 or 3 days in the human fetus. However, because the increase in left ventricular output was less than the decrease in right ventricular output, the combined ventricular output fell, presumably in shocklike response to the acute stress of ductal occlusion.

The cause of the regurgitation of the tricuspid valve in this setting remains unclear. Perhaps the sudden demand to generate higher pressures resulted in changes of the right ventricular myocardial architecture affecting the papillary muscles of the tricuspid valve. That tricuspid regurgitation immediately disappeared with afterload reduction supports this hypothesis. Ischemia as the cause of triscuspid regurgitation is very unlikely because the intervals were not long enough for ischemic changes to develop and because the tricuspid regurgitation was immediately reversible. Thus tricuspid regurgitation in the early phase of ductal constriction need not be irreversible. To what

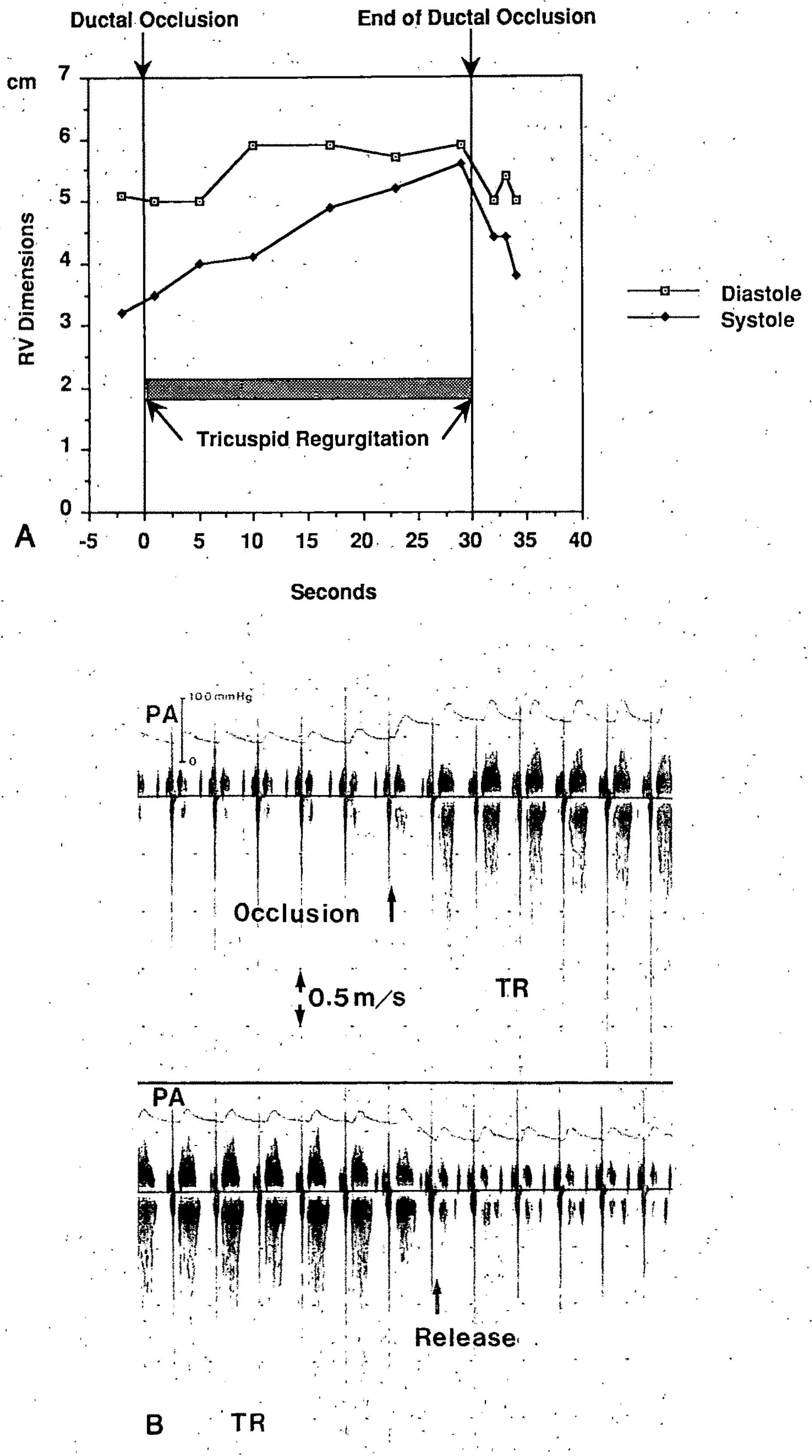

Fig. 2. Time course of fetal ductal occlusion. **A,** Right ventricular *(RV)* dimension by M-mode echocardiography during occlusion shows reversible increase in systolic and diastolic dimensions. **B,** Continuous-wave Doppler echocardiogram of tricuspid valve regurgitation *(TR)*, with onset within two beats after ductal occlusion and resolution after release of occlusion. Pulmonary artery pressure *(PA)* changes with ductal occlusion are also shown. *m/s,* Meters per second.

degree the right ventricle is able to adjust to a long-term increased afterload and whether the tricuspid valve becomes competent again after right ventricular adaptation have yet to be determined.

Acute ductal occlusion led to right ventricular dilatation and to a decreased right ventricular shortening fraction. These findings were easily recognizable during two-dimensional echocardiography. It is possible to diagnose ductal constriction in the human fetus; however, there has never been a documented case of ductal occlusion occurring in a human being. Whether decreased right ventricular shortening is a sign of ductal constriction and not occlusion has yet to be determined.

We concluded that immediate tricuspid regurgitation after acute ductal occlusion was reversible and appeared to be caused by right ventricular pressure increase rather than by ventricular dilatation. Major redistribution of flow occurred during fetal ductal occlusion, with an increase in left ventricular output and a decrease in right ventricular output. A dramatic change in right ventricular diameter and right ventricular shortening fraction accompanies fetal ductal occlusion.

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LETTERS TO THE EDITORS

Prognostic value of CA 125 and neopterin in women with ovarian cancer undergoing second-look laparotomy

To the Editors: We have read the article by Hunter et al. (Hunter VJ, Daly L, Helms M, et al. The prognostic significance of CA 125 half-life in patients with ovarian cancer who have received primary chemotherapy after surgical cytoreduction. AM J OBSTET GYNECOL 1990;163:1164-7) describing prolonged overall survival in patients with CA 125 half-life <20 days. The authors pointed out that after negative second-look procedures, "eight individuals whose serum CA 125 value normalized by 65 days after initial operation appeared to have improved survival." On the basis of a recent investigation,[1] we would like to extend the discussion raised by the authors.

We have determined serum concentrations of CA 125 and urine levels of neopterin immediately before second-look laparotomy in 72 women with ovarian cancer. Neopterin is a sensitive marker of cell-mediated immune activation.[2] One-year survival rates (mean ± SE) after surgical reexploration were associated ($p < 0.0001$) with concentrations of CA 125 (93% ± 4% in women with CA 125 values <35 U/ml; 47% ± 13% in women with higher CA 125 values) as well as neopterin (88% ± 5% in women with neopterin values <235 μmol/mol creatinine; 27% ± 13% in women with higher neopterin values).

Multivariate analysis was done to assess whether both predictors were independent of the surgical findings at second look, and separate survival curves were obtained by subdividing the group according to second-look outcome. CA 125 serum levels showed a stronger direct correlation than neopterin with the surgical findings at the procedure but failed to reach joint significance in addition to surgical outcome. In contrast, a significant correlation of raised neopterin levels with more unfavorable prognosis was found in patients with no evidence of disease and in those with residual tumors <1 cm. Women with tumors >1 cm uniformly had poor prognoses, as did patients with low versus high neopterin levels.

Identification of patients with negative second-look laparotomies who are at higher risk of disease progression would be helpful in ovarian cancer follow-up. Our results suggest that CA 125 provides an excellent predictor of the findings at second-look operation but does not provide predictive information for further follow-up in addition to the surgical outcome. In contrast, neopterin concentrations (which show a weaker correlation with the outcome of second look) do provide independent prognostic information and may be used to define risk groups. Our results are compatible with the background of both markers: whereas CA 125 is a classic tumor marker, neopterin is an indicator of cell-mediated immune activation and thus contributes information about a mechanism different from instantaneous tumor progression.

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Response declined

Free β-protein studies need confirmation

To the Editors: I was impressed by the article of Macri et al. (Macri JN, Kasturi RV, Krantz DA, et al. Maternal serum Down syndrome screening: Free β-protein is a more effective marker than human chorionic gonadotropin. AM J OBSTET GYNECOL 1990;163:1248-53). However, the following month an advertisement appeared in my mail over the senior author's signature and from his *commercial* laboratory. It extolled free β-protein as a "patent pending scientific discovery" that "is the most accurate and reliable biochemical marker for Down's syndrome screening," citing this article by Macri et al.

The JOURNAL asks potential authors about conflict of interest, and this appears to be an example of significant "commercial association of the author." In my view, the findings of Macri et al. should be quickly confirmed by an independent investigator.

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Reply

To the Editors: Dr. Goodlin is aware that it is conventional in clinical investigation to subject an original scientific discovery to retrospective studies, to publish these findings after peer review, and thereafter to undertake prospective studies. A careful reading of what Goodlin refers to as "an advertisement" reveals that it is actually a letter calling for participation in a prospective study. The letter states: "The discovery has been reduced to practice in an assay system and clinical reporting protocol for *investigational* Down syndrome screening" (emphasis added). Indeed, our Research Division has placed announcements concerning requests for proposals in major journals of obstetrics to advance additional free β-protein research and development.

We object to Goodlin's implication of conflict of interest in our publication of the effectiveness of free β-protein as a marker for Down syndrome. All original scientific contributions emanating from the Research Division of NTD Laboratories, Inc., have been clearly identified as such; the present case is no exception. Surely Dr. Goodlin would not suggest that research scientists with an explicit commercial association be denied the privilege of publication in the professional scientific literature or the benefits derived from peer review of original findings.

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Value of high and low maternal serum α-fetoprotein levels in screening singleton pregnancies

To the Editors: Milunsky et al. reported an important prospective study to establish the overall benefits of high, low, and normal maternal α-fetoprotein levels in screening singleton pregnancies (Milunsky A, Jick SS, Bruell CL, et al. Predictive values, relative risks and overall benefits of high and low maternal serum α-fetoprotein screening in singleton pregnancies: New epidemiologic data. AM J OBSTET GYNECOL 1989;161:291-7). We read with great interest this fundamental study, and we wish to comment on the relationship of high maternal serum α-fetoprotein levels and the incidence of fetal chromosome defects. In their Table I they have reported one chromosome anomaly associated with a high maternal serum α-fetoprotein level. In contrast, in their hierarchical Table III this case has not been reported. Furthermore, in their Table IV no probabilities for chromosome defects were given in relation to high maternal serum α-fetoprotein levels.

In our opinion chromosome disorders are hierarchically top-ranking obstetric events. Moreover, the possible relationship of high maternal serum α-fetoprotein levels and chromosome defects has attracted the scientific attention of several studies.[1, 2] If we consider the one case of chromosome disorder in the 530 cases of high maternal serum α-fetoprotein levels (Table I), then the risk for a woman with a high maternal serum α-fetoprotein level of carrying a fetus with a chromosome defect is 1 in 530. This incidence, without any adjustment for maternal age, fetal structural defects, and amniotic fluid α-fetoprotein levels, is comparable to the incidence of Down syndrome in live-born infants of woman 34 years old.[3]

We believe that this part of maternal serum α-fetoprotein screening needs more clarification.

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Reply

To the Editors: We appreciate the detailed reading of our article by Minaretzis and Tsionou, who correctly noted that the single chromosome anomaly reported in Table I is not shown in the hierarchical Table III. Further attention to the table footnotes would clarify their concern. The single chromosome anomaly has been incorporated in the neural tube defect group in the hierarchical table, each case being recorded only once, with neural tube defects taking precedence in being listed first. Table IV reflects this hierarchical tabulation as well and, hence, yielded no calculable odds for chromosome defects in this study in association with high maternal serum α-fetoprotein levels. Clearly, chromosome defects in the absence of any leaking lesions, such as spina bifida, omphalocele, and cystic hygroma, are very uncommon indeed. This observation is in line with those of others who have published on this association.

If Minaretzis and Tsionou wish to extrapolate from our data that 1 in 530 women had a chromosome defect, so be it. This figure would not come close to any standard recommendation for amniocentesis for prenatal genetic studies. In essence, fetal chromosome defects and raised maternal serum α-fetoprotein levels are a very infrequent association.

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Laparoscopic ventrosuspensions

To the Editors: I read with great interest the article by Yoong (Yoong AFE. Laparoscopic ventrosuspensions. AM J OBSTET GYNECOL 1990;163:1151-3). This point has been a concern of ours, and our team is quite used to uterine retroversion and its treatment.[1] Our results are better than Yoong's, perhaps because of the selected cases with which we deal. I have two questions: (1) Dr. Yoong does not specify whether uterine retroversion was observed in each case. (2) There is no mention of pelvic varices in Table III. Is this because none were sought?

Laparoscopic ventrosuspension is effective in treating severe dyspareunia and chronic pelvic pain. If the cause of the pain is anatomic, the efficacy of the procedure is more important in selected women.[1] To say that this procedure "does not appear to be helpful" seems excessive.

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REFERENCE

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Reply

To the Editors: It seems unlikely that any gynecologist would antevert an already anteverted uterus. The association between pelvic varicosities and pelvic pain is well known; had pelvic varicosities been seen, they would have been reported. Pelvic venography is necessary to confirm ovarian vein dilatation and slowed clearance of dye from the pelvis.[1, 2]

Analysis of the data collected did *not* prove the efficacy of the procedure: At least 53.5% of the women in the study felt no better 6 months after laparoscopic ventrosuspension. Gleeson and Gaffney[3] reached a similar conclusion.

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Laparoelytrotomy: Abdominal delivery without uterine incision

To the Editors: According to Garrigues,[1] extraperitoneal approach to the vagina to perform a cesarean section without a uterine incision was first proposed by Joerg in 1806. Several different modifications have been proposed and presented since then.[2, 3]

Under certain circumstances laparoelytrotomy may be inadvertently or, rarely, intentionally performed instead of a low-segment uterine cesarean section.[4] Goodlin[3] reported one intentional and four incidental laparoelytrotomies performed at Nebraska University Hospital in 1982. Our letter reports an incidental case of laparoelytrotomy.

A 23-year-old primigravid woman was at term and had been in the second stage of labor for approximately 3 hours with no analgesics. The vertex remained at 0 station in the left occipitotransverse position for 1½ hours. In the first stage of labor, when the cervix was dilated to 6 cm and 90% effaced, amniotomy had been performed.

The patient underwent a cesarean section because of the indication of cephalopelvic disproportion. With the patient under general anesthesia, the peritoneal cavity was entered. After the bladder flap was deflected downward and retained with a symphysis retractor, a low-transverse uterine incision was made and a 3700 gm male infant in good condition was delivered through the incision without difficulty. At the time of the incision we did not realize that the vagina rather than the lower uterine segment was being entered. When we tried to close the incision, we noted that the cervix was about 0.5 cm above the vaginal incision. The amount of bleeding was normal, and the only complication was a 0.5 cm laceration on the bladder wall with an intact bladder mucosa. This was easily repaired with 2-0 Dexon sutures. The patient was placed on a regimen of antibiotics, penicillin G procaine 800,000 U twice a day intramuscularly and gentamicin 80 mg three times a day intramuscularly.The urethral catheter was in place for 5 days. Patient and infant were discharged in good condition on the seventh postoperative day.

There is a tendency toward longer second stages of labor and avoidance of midforceps delivery. Cesarean sections after long second stages usually have higher morbidity than cesarean sections in uncomplicated cases. Cases like ours should be emphasized and discussed more in the literature so that complications can be decreased.

As Goodlin[3] mentioned, for the abdominal-vaginal approach to be effective, the cervix should be completely dilated, elevated, and retracted. The fetal vertex should be in the vagina and the vesicouterine fold should be freed further downward than in conventional cesarean sections. After the bladder is completely retracted, the vagina has a characteristic ballooned, shiny appearance.

If there is any doubt about landmarks, the fetus should be approached through the lower uterine segment through an incision on the upper side.

Another point is whether the scarred vagina may rupture or become an obstacle to labor. Further knowledge and discussion are necessary in this subject. A technically advanced laparoelytrotomy may become an alternative to conventional cesarean sections under certain circumstances.

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Prenatal prediction of Down syndrome

To the Editors: O'Brien et al. (O'Brien W, Knuppel R, Torres C, Sternlicht D. Potential prenatal predictions of Down syndrome: A statistical analysis. Am J Obstet Gynecol 1990;163:1796-8) showed that α-fetoprotein, human chorionic gonadotropin, and biparietal diameter/femur length ratio all are sufficiently independent to be used in combination for prenatal prediction of Down syndrome. This requires the use of multivariate gaussian statistics,[1] which have the property that any inaccuracies are multiplied. We statistically examined the screening data of 4605 women routinely screened for fetuses with Down syndrome. There were 2834 complete data sets: age at estimated date of confinement, last menstrual period, biparietal diameter (outer-to-outer table), femur length, α-fetoprotein, human chorionic gonadotropin, and blood sample collected on the same day as ultrasonographic scan.

We demonstrated, using ultrasonographic dating of gestational age expressed as weeks' gestation, that the true calculated risk based on two parameters (α-fetoprotein and human chorionic gonadotropin) may lie anywhere in the range 78% to 152% of the actual calculated risk (analytic errors ignored). Similarly, by use of three parameters (α-fetoprotein, human chorionic gonadotropin, and urinary estradiol), the true risk may lie in the range 40% to 250% of the actual risk (unpublished data). These errors are important because they may result in an incorrect decision about the requirement for amniocentesis and also because they are avoidable. The error is caused by the conversion of a relatively precise measure of fetal development (biparietal diameter) into an imprecise measure (weeks' gestation). Basing gestational age on last menstrual period also is not precise enough because of poor recall by patients and variability of menstrual cycle length.

O'Brien et al. showed a trend for biparietal diameter/femur length ratio to decrease between 15 and 18 weeks' gestation. However, they only provided details of the ratio by week of gestation. If biparietal diameter/femur length ratio is to be of use in screening, errors resulting from gestation dating must be avoided as far as possible; therefore we examined the relationship between the ratio and gestational stage assessed by raw biparietal diameter value and by ultrasonographically derived weeks' gestation. We also found a trend in ratio relative to weeks' gestation, but we found that at the edges of the biparietal diameter size band there were significant deviations from the ratio derived by using weeks' gestation to define fetal age.

We believe that using actual biparietal diameter and not biparietal diameter–derived weeks' gestation for assessment of gestational age will decrease edge effects, thereby improving the precision of data used to calculate risk. This should significantly improve screening for Down syndrome–associated pregnancy by reducing false-positive and false-negative screening results. Therefore we suggest that, instead of using week-based medians for determining expected biparietal diameter/femur length ratio, the following linear regression parameters (product moment) such that biparietal diameter/femur length ratio = A + (Biparietal diameter × B) should be used ($A = 1.97997$; $B = -0.0080335$; $r = -0.202$).

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REFERENCE

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Reply

To the Editors: I appreciate the interest the readers have demonstrated in our study and am pleased to see that their findings are similar to ours. I agree with the difficulties encountered in the use of multivariate gaussian statistics and agree that conversion of biparietal diameter (or femur length) measurements to gestational age assessment results in a loss of precision.

This conversion, however, allows consideration of several recent parameters in the same framework as the well-established reporting of α-fetoprotein. I suspect that, in spite of the mathematical superiority of computational values, as pointed out by the readers, practical consideration will lead to the use of weekly medians, as has occurred with α-fetoprotein testing.

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Is opposition to abortion unethical?

To the Editors: The "obstetric ethic" described by Chervenak and McCullough in the November 1990 issue of the JOURNAL (Chervenak FA, McCullough LB. Does obstetric ethics have any role in the obstetrician's response to the abortion controversy? AM J OBSTET GYNECOL 1990;163:1425-9) justifies abortion on demand before fetal viability by excluding any opposing arguments that are based on religious traditions.

Recently, however, a leading bioethicist noted that the biomedical ethics debate has been impoverished by the a priori exclusion of arguments that are based on various religious traditions.[1] In fact, all arguments for and against unrestricted abortion are based on fundamental presuppositions of some type. Whether they are religious is irrelevant.

My question to the authors of this Clinical Opinion is, in light of the fundamental presuppositions of their "obstetric ethic," is it unethical for an obstetrician to oppose the current practice of abortion on demand?

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REFERENCE

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Reply

To the Editors: Dr. Calvin uses a phrase that is used commonly in the abortion controversy but also has many meanings. "Abortion on demand" can mean that a physician is obligated to perform an abortion even if doing so, for whatever reason, violates the physician's private conscience. We argue explicitly that there is no such ethical obligation. Moreover, we point out that religious traditions are a primary influence on the formation of private conscience. Thus we can in no way be understood to exclude altogether religious traditions from obstetric ethics.

There is a second sense of "abortion on demand"—a phrase we do not use because we find it useless—that means that there can be no objections to abortion because the fetus never had moral status. We argue that the fetus does indeed sometimes have the moral status of being a patient. Hence we would reject "abortion on demand" in this second sense of the phrase.

In answer to Dr. Calvin's question, we stated that, either for reasons of private conscience or when the fetus is a patient, any obstetrician should have grave moral reservations about "abortion on demand" in the above two senses.

There is yet a third sense of "abortion on demand," namely, that pregnant women should be free to withhold, confer, or, having once conferred, withdraw the moral status of being a patient on the previable fetus. In effect, our article defends this sense of "abortion on demand," even while rejecting the previous senses of the phrase.

Frank A. Chervenak, MD

The New York Hospital–Cornell Medical Center, 525 East 68th St.-M713, New York, NY 10021

Laurence B. McCullough, PhD

Center for Ethics, Medicine, and Public Issues, Departments of Medicine and Community Medicine, Baylor College of Medicine, 6550 Fannin, Houston, TX 77030

Examination of placentas by pathologists

To the Editors: The opinions expressed by Salafia and Vintzileos (Salafia CM, Vintzileos AM. Why all placentas should be examined by a pathologist in 1990. AM J OBSTET GYNECOL 1990;163:1282-93) represent wishful thinking regarding the ability of a pathologist's examination of the placenta to give meaningful information not better obtained by clinical means.

In the comment section of their article they imply that examination of the placenta can "identify the exact cause" of an untoward outcome of pregnancy. However, numerous studies, including a major contribution among their own references, have emphasized the relative nonspecificity of pathologic examination, both gross and microscopic.[1] On the contrary, the experienced pathologist knows the disappointment of reporting, for example, severe chorioamnionitis by microscopy to a colleague, only to learn that the product of the suspect pregnancy was a bouncingly healthy neonate.

Furthermore, their Table IV lists findings and their respective possible causes, for which one can easily suggest superior clinical or laboratory means to make the diagnosis.

Finally, I find their cost-effectiveness argument quite unconvincing: To generate a charge of $2 million as prophylaxis against a possible $5 to $6 million lawsuit is not powerful leveraging. A cost of $225 per placenta for useless information is unconscionable in this time of diminished medical resources and merely buys the obstetrician a false sense of added security.

Howard Goldman, MD

Department of Pathology, Merrithew Memorial Hospital, 2500 Alhambra Ave., Martinez, CA 94553

REFERENCE

1. Fox H. Pathology of the placenta. In: Bennington JL, ed. Major problems in pathology. Philadelphia: WB Saunders, 1978 vol 7.

Reply

To the Editors: Unfortunately Dr. Goldman has missed our point. This is evident from his first paragraph. We did not represent placental pathologic examination to stand independent of, separate from, or as a substitute for clinical means of obstetric assessment. We instead emphasized that a pathologist requires clinical information to understand the relevance of placental lesions to outcome. Referencing a text does not require that we accept all its tenets, and we have significant disagreements with Dr. Fox in many areas. We are amazed that Dr. Goldman could be disappointed by reporting severe chorioamnionitis in the face of a "bouncingly healthy" neonate. Acute ascending infections may have a multiplicity of effects in the fetus-neonate that are remote from sepsis. The pathologist and the obstetrician should communicate on each case; the obstetrician should recognize that subclinical chorioamnionitis caused fetal distress,[1, 2] which led to intervention and, thankfully, a healthy newborn. Only then will the pathologist recognize that chorioamnionitis may be clinically significant in the absence of infectious morbidity or mortality. Dr. Goldman apparently fails to consider that a healthy newborn does not of necessity grow up to be a neurologically normal child. As we cited, 85% of cases of mental retardation have no scientific or clinically based explanation.[3] Experimental and clinical studies have shown that both acute intraamniotic infection and chronic villitis representing a potential congenital viral infection can be related in a causal fashion to long-term neurologic impairment.[4,5] More than 75% of histologically evident intraamniotic infection ("chorioamnionitis") and >95% of cases of chronic villitis are clinically silent, we submit that the "usefulness" of the information derived from placental pathologic examination will be minimal if the only outcome variable to be considered is the level of neurologic function at birth. In this discussion we also indirectly addressed the issues raised in the third paragraph. Chronic villitis, hemorrhagic endovillitis, infarcts, and other villous pathologic conditions cannot be definitely assessed by any modality other than placental examination. We stand by our assertion that placental examination *may* identify the exact cause of poor pregnancy outcome. Finally, we agree that the cost of placental pathologic examination, if the information is not combined with the obstetric clinical data, is indeed unconscionable. Placental pathologic examination cannot be considered to be separate from, or a substitute for, clinical assessment and only achieves its optimal returns when utilized in concert with clinical and laboratory investigations. Our article was born of the realization that the unique benefits we obtained from placental examinations in our institution were derived from our communications and interactions on the obstetrics floor, in the delivery suite, and at morbidity and mortality reviews.

We thank Dr. Goldman for his comments. He has permitted us to restate, perhaps more strongly, the need for *informed* examination of the placenta, informed by clinical information and by collaboration with obstetric colleagues.

Carolyn M. Salafia, MD, and Anthony M. Vintzileos, MD
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, The University of Connecticut Health Center, Farmington, CT 06032

REFERENCES

1. Salafia CM, Mangam HE, Weigl CA, Foye GJ, Silberman L. Abnormal fetal heart rate pattern and placental inflammation. AM J OBSTET GYNECOL 1989;160:140.
2. Fleming AD, Salafia CM, Vintzileos AM, Rodis JF, Campbell WA, Bantham KF. The relationship among umbilical artery velocimetry, fetal biophysical profile, and placental inflammation in preterm premature rupture of the membranes. AM J OBSTET GYNECOL 1991;164:38-41.
3. Mann LI. Pregnancy events and brain damage. AM J OBSTET GYNECOL 1986;155:6-9.
4. Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. 3rd ed. Philadelphia: WB Saunders, 1990:774.
5. Remington JS, Klein JO, eds. Infectious diseases of the fetus and newborn infant. 3rd ed. Philadelphia: WB Saunders, 1990:262.

Maternal genital herpes and gender of offspring

To the Editors: In their Letter to the Editors McGregor and Leff (McGregor JA, Leff M. Does maternal genital herpes infection influence fetal gender? AM J OBSTET GYNECOL 1990;162:1346-7) suggest that there is a decreased proportion of male infants born to mothers with a history of genital herpes complicating pregnancy. This caused us to review the data from our cohort of pregnant women with symptomatic and asymptomatic genital herpes at the University of Washington. Among women with herpesvirus type 2 type-specific antibodies, as detected by Western blot analysis, we demonstrated a normal male/female ratio of their offspring (Table I).

Table I. Maternal herpesvirus serologic results and gender of offspring ($N = 2084$)

Infants	*Seronegative*	*Herpesvirus type 1*	*Herpesvirus type 2*	*Herpesvirus types 1 and 2*
Male	234 (50.6%)	503 (50.9%)	169 (53.7%)	162 (50.8%)
Female	228 (49.4%)	485 (49.1%)	146 (46.3%)	157 (49.2%)

$\chi^2 = 0.87$; $p = .83$.

Table II. Maternal herpesvirus history and gender of offspring ($N = 2041$)

Infants	*No history*	*Herpesvirus before pregnancy*	*Herpesvirus during pregnancy*
Male	943 (51.7%)	86 (48%)	21 (53.8%)
Female	880 (48.3%)	93 (52%)	18 (46.2%)

$\chi^2 = 0.97$; $p = 0.61$.

Similarly, in this same cohort, when we calculated the percentage of male infants on the basis of the clinical history rather than serologic results, again there was no alteration in the normal male/female ratio among offspring (Table II).

Zane Brown, MD, and Larry Corey, MD
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, RH-20, University of Washington School of Medicine, Seattle, WA 98195

Reply

To the Editors: My colleagues and I are pleased that Brown and Corey have answered our question in their uniquely well-described population. As noted in our letter, others have described fetal and newborn gender differences among women with proven or possible perinatal infectious processes in a variety of settings; male gender consistently predisposes to perinatal infection in studies going back decades.[1-3] MacGillivray and Davey[3] noted convincingly higher rates of preterm birth and premature rupture of membranes among South African women giving birth to male infants. Conversely, in a recent analysis of 8758 births at the University of Colorado, similar risks of preterm premature rupture of membranes, clinical chorioamnionitis, and postpartum endometritis were found in women giving birth to male and female infants.[4] These contrasting findings suggest that male gender–linked predispositions to perinatal infection are most likely to be expressed in women at high risk for reproductive tract infections before or during pregnancy. Controlled studies of possible interactions of reproductive tract infection, fetal gender, and the pathologic characteristics of parturition are required to fully examine such suggestions. My colleagues and I thank Brown and Corey for their observations.

James A. McGregor, MD, CM

Department of Obstetrics and Gynecology, University of Colorado Health Sciences Center, 4200 East Ninth Ave., Denver, CO 80262

REFERENCES

1. Washburn TC, Medearis DNJ, Childs B. Sex differences in susceptibility to infection. Pediatrics 1965;35:57-64.
2. St Geme JN Jr, Murray DL, Carter J, et al. Perinatal bacterial infection after prolonged rupture of amniotic membrane: an analysis of risk and management. J Pediatr 1984;104:608-13.
3. MacGillivray I, Davey DA. The influence of fetal sex on rupture of membranes and preterm labor (Letter). AM J OBSTET GYNECOL 1985;153:814-5.
4. McGregor JA, Leff M, Orleans M, Baron A. Fetal gender differences in preterm birth: findings in a North American cohort. Am J Perinatol [In press].

Correction

In the article by Weiner et al., entitled "The therapeutic efficacy and cost-effectiveness of aggressive tocolysis for premature labor associated with premature rupture of the membranes" (AM J OBSTET GYNECOL 1988;159:216-22), in Table V on page 219, the second major heading in the left-hand column was omitted. The correct table is shown here.

Table V. Comparisons by selected intervals

	Bed rest (%)	*Tocolysis (%)*
From membrane rupture to delivery		
<24 hr	7.1	6.1
<48 hr	23.8	12.1
<72 hr	38.1	30.3
From onset of regular contractions to delivery		
<24 hr	42.1	24.1
<48 hr	63.2	55.1
<72 hr	71.1	62.1

All differences nonsignificant.

ITEMS

Announcements of major meetings and other significant activities must be received at least 8 weeks before the desired month of publication. All announcements carry a charge of $60.00 U.S. per insertion and the fee must accompany the request to publish. Information will be limited to title of meeting, date, place, and an address to obtain further information. Send announcements and payment, payable to this JOURNAL, to Kay G. Goehler, Senior Manuscript Editor, Journal Editing, Mosby–Year Book, Inc., 11830 Westline Industrial Drive, St. Louis, MO 63146-3318.

Obstetric/Pelvic Imaging, Hands-on Instruction in Diagnostic Ultrasound. Conducted October 21-25, 1991, in Dallas, Texas. For further information call or write Keith Mauney & Associates, Inc., 600 E. Las Colinas Blvd., Suite 250, Irving, TX 75039; (214) 402-8250.

Royal College of Obstetricians & Gynaecologists. The 26th British Congress of Obstetrics & Gynaecology will take place at the University of Manchester Institute of Science and Technology, Manchester, England, from July 7-10, 1992. Information available from BCOG Secretariat, 65 West Dr., Cheam, Sutton, Surrey, SM2 7NB, UK.

The IXth International Congress of Endocrinology, Acropolis, Convention Center, Nice, France, August 30 to September 5, 1992. For information contact: IXth International Congress of Endocrinology, SOCFI, 14, Rue Mandar, 75002 Paris, France. Tel.: 33 (1) 42 33 89 94. Fax: 33 (1) 40 26 04 44.

Second International Capri Conference. The Second International Capri Conference will be held in Capri, Italy, May 23-26, 1992, by Conference Presidente U. Montomagno, A.R. Genazzani, and C. Nappi. The program will include symposia, round tables, free communications, and posters devoted to "Neuroendocrine disorders of female reproductive system: Pathophysiology and therapies." Deadline for abstract submission is February 28, 1992. For further information please contact: Prof. C. Nappi, Department of Obstetrics and Gynaecology, Univ. of Napoli, V. Pansini 5, 80131 Naples, Italy. Tel.: +39 81 7462979. Fax: +39 81 7462903. Or Dr. F. Petraglia, Department of Obstetrics and Gynaecology, Univ. of Modena, V. del Pozzo 71, 41100 Modena, Italy. Tel.: +39 59 379123. Fax: +39 59 371401.

Gynecology Update 1991, presented by Grossmont Hospital's Womens' Center, Saturday, Nov. 9, 1991, at the San Diego Marriott at the Harbor. For information and registration, please contact Christine Hardiman, Womens' Health Center, Grossmont Hospital, P.O. Box 158, La Mesa, CA 91944. Tel.: (619) 589-4951.

Ninth Annual Ella T. Grasso Memorial Conference, Wednesday, November 20, 1991, The Park Plaza, 155 Temple St., New Haven, Connecticut. This symposium addresses clinical management, therapy, and new advances in gynecologic oncology and is directed toward obstetricians/gynecologists, medical oncologists, radiation therapists, pathologists, nurses, and residents. Sponsors: Division of Gynecologic Oncology of Yale University School of Medicine, Yale Comprehensive Cancer Center, and University of Connecticut School of Medicine. For further information and registration contact: Postgraduate and Continuing Medical Education, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06510-8052. Tel.: (203) 785-4578.

Problem Solving in Gynecologic Endocrinology and Infertility, December 13-14, 1991, The Hotel Inter-Continental, Chicago, Illinois. Credits: 10 Hours, Category 1, 10 Cognates, Formal Learning, ACOG. For information contact: The University of Chicago, Center for Continuing Medical Education, 5841 South Maryland, Box 139, Chicago, IL 60637. Tel.: (312) 702-1056.

DIRECTOR OF MATERNAL-FETAL MEDICINE

Albert Einstein Medical Center (AEMC), a 600+ bed tertiary care teaching hospital, is seeking a board certified maternal-fetal medicine subspecialist as Director of the Division of Maternal-Fetal Medicine to develop and direct the comprehensive perinatal program in this institution. AEMC is a major referral center in north Philadelphia supporting numerous post-graduate medical education programs, including a fully accredited residency program in obstetrics and gynecology. The Department of Obstetrics and Gynecology has a long-standing tradition of excellence in health-care delivery and supports a variety of clinical research and educational activities. The Division of Maternal-Fetal Medicine serves as a tertiary referral center within the metropolitan Philadelphia area. Its service includes maternal intensive care unit, maternal transport program, high risk obstetrics, genetic counseling, diagnostic genetic procedures (amniocentesis, CVS, cordocentesis). targeted (level II) obstetrical ultrasound and comprehensive antenatal fetal surveillance. Our Department is supported by a Level III NICU and a Cytogenetic Laboratory. There is a strong institutional support and emphasis in research with funds and laboratory space available for basic research. A construction project is under way for a new labor and delivery suite and a comprehensive fetal diagnostic center. We are also planning to develop a maternal-fetal medicine fellowship training program with Temple University.

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Symposia Medicus, 1299 Newell Hill Place, Suite 301, Walnut Creek, CA 94596-5220

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c/o Mark Gibson, M.D.
Professor and Chairman
Department of Obstetrics and Gynecology
West Virginia University School of Medicine
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Please send Curriculum Vitae to:

Eleanor L. Capeless, MD
Director, Maternal Fetal Medicine
Dept. of Ob/Gyn
Medical Center Hospital of Vermont
Burlington, VT 05401

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Box 395 UMHC
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Michael T. Mennuti, M.D.
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Department of Obstetrics
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Ian H. Thorneycroft, Ph.D., M.D.
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Search Chairperson
University of Minnesota
Department of Obstetrics and Gynecology
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IMPORTANT NOTE—This information is a BRIEF SUMMARY of the complete prescribing information provided with the product and therefore should not be used as the basis for prescribing the product. This summary was prepared by deleting from the complete prescribing information certain text, tables and references. The physician should be thoroughly familiar with the complete prescribing information before prescribing the product.

INDICATIONS AND USAGE: PREVENTION OF PREGNANCY.

CONTRAINDICATIONS: Oral contraceptives should not be used in women who currently have the following conditions: 1. Thrombophlebitis or thromboembolic disorders. 2. A past history of deep vein thrombophlebitis or thromboembolic disorders. 3. Cerebral vascular or coronary artery disease. 4. Known or suspected carcinoma of the breast. 5. Carcinoma of the endometrium or other known or suspected estrogen-dependent neoplasia. 6. Undiagnosed abnormal genital bleeding. 7. Cholestatic jaundice of pregnancy or jaundice with prior pill use. 8. Hepatic adenomas or carcinomas. 9. Known or suspected pregnancy.

WARNINGS

Cigarette smoking increases the risk of serious cardiovascular side effects from oral contraceptive use. This risk increases with age and with heavy smoking (15 or more cigarettes per day) and is quite marked in women over 35 years of age. Women who use oral contraceptives should be strongly advised not to smoke.

The use of oral contraceptives is associated with increased risks of several serious conditions including myocardial infarction, thromboembolism, stroke, hepatic neoplasia, and gallbladder disease, although the risk of serious morbidity or mortality is very small in healthy women without underlying risk factors. The risk of morbidity and mortality increases significantly in the presence of other underlying risk factors such as hypertension, hyperlipidemias, obesity and diabetes. Practitioners prescribing oral contraceptives should be familiar with the following information relating to these risks. The information contained in this brief summary is principally based on studies carried out in patients who used oral contraceptives with higher formulations of estrogens and progestogens than those in common use today. The effect of long term use of the oral contraceptives with lower formulations of both estrogens and progestogens remains to be determined. Throughout this brief summary epidemiological studies reported are of two types: retrospective or case control studies and prospective or cohort studies. Case control studies provide a measure of the relative risk of a disease, namely a *ratio* of the incidence of a disease among oral contraceptive users to that among nonusers. The relative risk does not provide information on the actual clinical occurrence of a disease. Cohort studies provide a measure of attributable risk, which is the *difference* in the incidence of disease between oral contraceptive users and nonusers. The attributable risk does provide information about the actual occurrence of a disease in the population. For further information, the reader is referred to a text on epidemiological methods. 1. THROMBOEMBOLIC DISORDERS AND OTHER VASCULAR PROBLEMS. a. Myocardial Infarction. An increased risk of myocardial infarction has been associated with oral contraceptive use. This risk is primarily in smokers or women with other underlying risk factors for coronary artery disease such as hypertension, hypercholesterolemia, morbid obesity, and diabetes. The relative risk of heart attack for current oral contraceptive users has been estimated to be two to six. The risk is very low under the age of 30. Smoking in combination with oral contraceptive use has been shown to contribute substantially to the incidence of myocardial infarctions in women in their mid-thirties or older with smoking accounting for the majority of excess cases. Mortality rates associated with circulatory disease have been shown to increase substantially in smokers, especially in those 35 years of age and older among women who use oral contraceptives. Oral contraceptives may compound the effects of well-known risk factors, such as hypertension, diabetes, hyperlipidemias, age and obesity. In particular, some progestogens are known to decrease HDL cholesterol and cause glucose intolerance, while estrogens may create a state of hyperinsulinism. Oral contraceptives have been shown to increase blood pressure among users (see section 9 in WARNINGS). Similar effects on risk factors have been associated with an increased risk of heart disease. Oral contraceptives must be used with caution in women with cardiovascular disease risk factors. b. Thromboembolism. An increased risk of thromboembolic and thrombotic disease associated with the use of oral contraceptives is well established. Case control studies have found the relative risk of users compared to non-users to be 3 for the first episode of superficial venous thrombosis, 4 to 11 for deep vein thrombosis or pulmonary embolism, and 1.5 to 6 for women with predisposing conditions for venous thromboembolic disease. Cohort studies have shown the relative risk to be somewhat lower, about 3 for new cases and about 4.5 for new cases requiring hospitalization. The risk of thromboembolic disease associated with oral contraceptives is not related to length of use and disappears after pill use is stopped. A two- to four-fold increase in relative risk of post-operative thromboembolic complications has been reported with the use of oral contraceptives. The relative risk of venous thrombosis in women who have predisposing conditions is twice that of women without such medical conditions. If feasible, oral contraceptives should be discontinued at least four weeks prior to and for two weeks after elective surgery of a type associated with an increase in risk of thromboembolism and during and following prolonged immobilization. Since the immediate postpartum period is also associated with an increased risk of thromboembolism, oral contraceptives should be started no earlier than four weeks after delivery in women who elect not to breast feed. c. Cerebrovascular diseases. Oral contraceptives have been shown to increase both the relative and attributable risks of cerebrovascular events (thrombotic and hemorrhagic strokes), although, in general, the risk is greatest among older (>35 years), hypertensive women who also smoke. Hypertension was found to be a risk factor for both users and non-users, for both types of strokes, and smoking interacted to increase the risk of stroke. In a large study, the relative risk of thrombotic strokes has been shown to range from 3 for normotensive users to 14 for users with severe hypertension. The relative risk of hemorrhagic stroke is reported to be 1.2 for non-smokers who used oral contraceptives, 2.6 for smokers who did not use oral contraceptives, 7.6 for smokers who used oral contraceptives, 1.8 for normotensive users and 25.7 for users with severe hypertension. The attributable risk is also greater in older women. d. Dose-related risk of vascular disease from oral contraceptives. A positive association has been observed between the amount of estrogen and progestogen in oral contraceptives and the risk of vascular disease. A decline in serum high density lipoproteins (HDL) has been reported with many progestational agents. A decline in serum high density lipoproteins has been associated with an increased incidence of ischemic heart disease. Because estrogens increase HDL cholesterol, the net effect of an oral contraceptive depends on a balance achieved between doses of estrogen and progestogen and the activity of the progestogen used in the contraceptive. The activity and amount of both hormones should be considered in the choice of an oral contraceptive. Minimizing exposure to estrogen and progestogen is in keeping with good principles of therapeutics. For any particular estrogen/progestogen combination, the dosage regimen prescribed should be one which contains the least amount of estrogen and progestogen that is compatible with a low failure rate and the needs of the individual patient. New acceptors of oral contraceptive agents should be started on preparations containing 0.035 mg or less of estrogen. e. Persistence of risk of vascular disease. There are two studies which have shown persistence of risk of vascular disease for ever-users of oral contraceptives. In a study in the United States, the risk of developing myocardial infarction after discontinuing oral contraceptives persists for at least 9 years for women 40-49 years who had used oral contraceptives for five or more years, but this increased risk was not demonstrated in other age groups. In another study in Great Britain, the risk of developing cerebrovascular disease persisted for at least 6 years after discontinuation of oral contraceptives, although excess risk was very small. However, both studies were performed with oral contraceptive formulations containing 50 micrograms or higher of estrogens. 2. ESTIMATES OF MORTALITY FROM CONTRACEPTIVE USE. One study gathered data from a variety of sources which have estimated the mortality rate associated with different methods of contraception at different ages. These estimates include the combined risk of death associated with contraceptive methods plus the risk attributable to pregnancy in the event of method failure. Each method of contraception has its specific benefits and risks. The study concluded that with the exception of oral contraceptive users 35 and older who smoke and 40 and older who do not smoke, mortality associated with all methods of birth control is low and below that associated with childbirth. However, smokers 35 and older and non-smokers 40 and older who use oral contraceptives have a significant increase in mortality higher than those using other methods of birth control. These facts must be weighed in conjunction with failure rates for other methods and the risk associated with subsequent pregnancy. 3. CARCINOMA OF THE REPRODUCTIVE ORGANS. Numerous epidemiological studies have been performed on the incidence of breast, endometrial, ovarian and cervical cancer in women using oral contraceptives. While there are conflicting reports most studies suggest that use of oral contraceptives is not associated with an overall increase in the risk of developing breast cancer. Some studies have reported an increased relative risk of developing breast cancer, particularly at a younger age. This increased relative risk appears to be related to duration of use. Some studies suggest that oral contraceptive use has been associated with an increase in the risk of cervical intraepithelial neoplasia in some populations of women. However, there continues to be controversy about the extent to which such findings may be due to differences in sexual behavior and other factors. 4. HEPATIC NEOPLASIA. Benign hepatic adenomas are associated with oral contraceptive use, although the incidence of benign tumors is rare in the United States. Indirect calculations have estimated the attributable risk to be in the range of 3.3 cases/100,000 for users, a risk that increases after four or more years of use especially with oral contraceptives of higher dose. Rupture of benign, hepatic adenomas may cause death through intra-abdominal hemorrhage. Studies from Britain have shown an increased risk of developing hepatocellular carcinoma in long-term (>8 years) oral contraceptive users. However, these cancers are rare in the U.S. and the attributable risk (the excess incidence) of liver cancers in oral contraceptive users approaches less than one per million users. 5. OCULAR LESIONS. There have been clinical case reports of retinal thrombosis associated with the use of oral contraceptives. Oral contraceptives should be discontinued if there is unexplained partial or complete loss of vision; onset of proptosis or diplopia, papilledema, or retinal vascular lesions. Appropriate diagnostic and therapeutic measures should be undertaken immediately. 6. ORAL CONTRACEPTIVE USE BEFORE OR DURING EARLY PREGNANCY. Extensive epidemiological studies have revealed no increased risk of birth defects in women who have used oral contraceptives prior to pregnancy. The majority of recent studies also do not indicate a teratogenic effect, particularly in so far as cardiac anomalies and limb reduction defects are concerned, when taken inadvertently during early pregnancy. The administration of oral contraceptives to induce withdrawal bleeding should not be used as a test for pregnancy. Oral contraceptives should not be used during pregnancy to treat threatened or habitual abortion. It is recommended that for any patient who has missed two consecutive periods pregnancy should be ruled out before continuing oral contraceptive use. If the patient has not adhered to the prescribed schedule, the possibility of pregnancy should be considered at the time of the first missed period. Oral contraceptive use should be discontinued until pregnancy is ruled out. 7. GALLBLADDER DISEASE. Earlier studies have reported an increased lifetime relative risk of gallbladder surgery in users of oral contraceptives and estrogens. More recent studies, however, have shown that the relative risk of developing gallbladder disease among oral contraceptive users may be minimal. The recent findings of minimal risk may be related to the use of oral contraceptive formulations containing lower hormonal doses of estrogens and progestogens. 8. CARBOHYDRATE AND LIPID METABOLIC EFFECTS. Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. This effect has been shown to be directly related to estrogen dose. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents. However, in the non-diabetic woman, oral contraceptives appear to have no effect on fasting blood glucose. Because of these demonstrated effects prediabetic and diabetic women in particular should be carefully monitored while taking oral contraceptives. A small proportion of women will have persistent hypertriglyceridemia while on the pill. As discussed earlier (see WARNINGS 1a and 1d), changes in serum triglycerides and lipoprotein levels have been reported in oral contraceptive users. 9. ELEVATED BLOOD PRESSURE. An increase in blood pressure has been reported in women taking oral contraceptives, and this increase is more likely in older oral contraceptive users and with extended duration of use. Data from the Royal College of General Practitioners and subsequent randomized trials have shown that the incidence of hypertension increases with increasing progestational activity. Women with a history of hypertension or hypertension-related diseases, or renal disease, should be encouraged to use another method of contraception. If women elect to use oral contraceptives, they should be monitored closely and if significant elevation of blood pressure occurs, oral contraceptives should be discontinued. For most women, elevated blood pressure will return to normal after stopping oral contraceptives, and there is no difference in the occurrence of hypertension between former and never users. 10. HEADACHE. The onset or exacerbation of migraine or development of headache with a new pattern which is recurrent, persistent or severe requires discontinuation of oral contraceptives and evaluation of the cause. 11. BLEEDING IRREGULARITIES. Breakthrough bleeding and spotting are sometimes encountered in patients on oral contraceptives, especially during the first three months of use. Non-hormonal causes should be considered and adequate diagnostic measures taken to rule out malignancy or pregnancy in the event of breakthrough bleeding as in the case of any abnormal vaginal bleeding. If pathology has been excluded, time or a change to another formulation may solve the problem. In the event of amenorrhea, pregnancy should be ruled out. Some women may encounter post-pill amenorrhea or oligomenorrhea, especially when such a condition was preexistent. 12. ECTOPIC PREGNANCY. Ectopic as well as intrauterine pregnancy may occur in contraceptive failures. **PRECAUTIONS:** 1. PHYSICAL EXAMINATION AND FOLLOW UP. A complete medical history and physical examination should be taken prior to the initiation or reinstitution of oral contraceptives and at least annually during use of oral contraceptives. These physical examinations should include special reference to blood pressure, breasts, abdomen and pelvic organs, including cervical cytology, and relevant laboratory tests. In case of undiagnosed persistent or recurrent abnormal vaginal bleeding, appropriate diagnostic measures should be conducted to rule out malignancy. Women with a strong family history of breast cancer or who have breast nodules should be monitored with particular care. 2. LIPID DISORDERS. Women who are being treated for hyperlipidemias should be followed closely if they elect to use oral contraceptives. Some progestogens may elevate LDL levels and may render the control of hyperlipidemias more difficult. 3. LIVER FUNCTION. If jaundice develops in any woman receiving such drugs, the medication should be discontinued. Steroid hormones may be poorly metabolized in patients with impaired liver function. 4. FLUID RETENTION. Oral contraceptives may cause some degree of fluid retention. They should be prescribed with caution, and only with careful monitoring, in patients with conditions which might be aggravated by fluid retention. 5. EMOTIONAL DISORDERS. Women with a history of depression should be carefully observed and the drug discontinued if depression recurs to a serious degree. 6. CONTACT LENSES. Contact lens wearers who develop visual changes or changes in lens tolerance should be assessed by an ophthalmologist. 7. DRUG INTERACTIONS. Reduced efficacy and increased incidence of breakthrough bleeding and menstrual irregularities have been associated with concomitant use of rifampin. A similar association, though less marked, has been suggested with barbiturates, phenylbutazone, phenytoin sodium, and possibly with griseofulvin, ampicillin and tetracyclines. 8. INTERACTIONS WITH LABORATORY TESTS. Certain endocrine and liver function tests and blood components may be affected by oral contraceptives: a. Increased prothrombin and factors VII, VIII, IX, and X; decreased antithrombin 3, increased norepinephrine-induced platelet aggregability. b. Increased thyroid binding globulin (TBG) leading to increased circulating total thyroid hormone, as measured by protein-bound iodine (PBI), T4 by column or by radio-immunoassay. Free T3 resin uptake is decreased, reflecting the elevated TBG, free T4 concentration is unaltered. c. Other binding proteins may be elevated in serum. d. Sex-binding globulins are increased and result in elevated levels of total circulating sex steroids and corticoids; however, free or biologically active levels remain unchanged. e. Triglycerides may be increased. f. Glucose tolerance may be decreased. g. Serum folate levels may be depressed by oral contraceptive therapy. This may be of clinical significance if a woman becomes pregnant shortly after discontinuing oral contraceptives. 9. CARCINOGENESIS. See WARNINGS section. 10. PREGNANCY. Pregnancy Category X. See CONTRAINDICATIONS and WARNINGS sections. 11. NURSING MOTHERS. Small amounts of oral contraceptive steroids have been identified in the milk of nursing mothers and a few adverse effects on the child have been reported, including jaundice and breast enlargement. In addition, oral contraceptives given in the postpartum period may interfere with lactation by decreasing the quantity and quality of breast milk. If possible, the nursing mother should be advised not to use oral contraceptives but to use other forms of contraception until she has completely weaned her child. **INFORMATION FOR THE PATIENT: See Patient Package Insert.**

ADVERSE REACTIONS: An increased risk of the following serious adverse reactions has been associated with the use of oral contraceptives (see WARNINGS section). Thrombophlebitis and venous thrombosis with or without embolism. Arterial thromboembolism. Pulmonary embolism. Myocardial infarction. Cerebral hemorrhage. Cerebral thrombosis. Hypertension. Gallbladder disease. Hepatic adenomas or benign liver tumors. The following adverse reactions have been reported in patients receiving oral contraceptives and are believed to be drug-related: Nausea. Vomiting. Gastrointestinal symptoms (such as abdominal cramps and bloating). Breakthrough bleeding. Spotting. Change in menstrual flow. Amenorrhea. Temporary infertility after discontinuation of treatment. Edema. Melasma which may persist. Breast changes: tenderness, enlargement, secretion. Change in weight (increase or decrease). Change in cervical erosion and secretion. Diminution in lactation when given immediately postpartum. Cholestatic jaundice. Migraine. Rash (allergic). Mental depression. Reduced tolerance to carbohydrates. Vaginal candidiasis. Change in corneal curvature (steepening). Intolerance to contact lenses. The following adverse reactions have been reported in users of oral contraceptives and the association has been neither confirmed nor refuted: Pre-menstrual syndrome. Cataracts. Changes in appetite. Cystitis-like syndrome. Headache. Nervousness. Dizziness. Hirsutism. Loss of scalp hair. Erythema multiforme. Erythema nodosum. Hemorrhagic eruption. Vaginitis. Porphyria. Impaired renal function. Hemolytic uremic syndrome. Acne. Changes in libido. Colitis.

OVERDOSAGE: Serious ill effects have not been reported following acute ingestion of large doses of oral contraceptives by young children. Overdosage may cause nausea and withdrawal bleeding may occur in females.

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†Serious as well as minor side effects have been reported with the use of oral contraceptives. The physician should remain alert to the earliest manifestations of any symptoms of serious disease and discontinue oral contraceptive therapy when appropriate.

‡Oral contraceptives have been shown to cause a decrease in glucose tolerance in a significant percentage of users. Progestogens increase insulin secretion and create insulin resistance, this effect varying with different progestational agents.

§An increase in blood pressure has been reported in women taking oral contraceptives.

1. Spellacy WN, Ellingson AB, Tsibris JCM. Glucose and insulin levels after six months of treatment with a triphasic oral contraceptive containing ethinyl estradiol and norethindrone. *J Reprod Med.* 1989;34(8):540-542.

Please see complete Prescribing Information, a brief summary of which is on the preceding page.

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OB-GYN CARE IN THE 1990s: THE CHLAMYDIA CHALLENGE

San Francisco, California
May 6, 1990

Chairman
Sebastian Faro, MD, PhD

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OB-GYN CARE IN THE 1990s: THE CHLAMYDIA CHALLENGE

Based on a symposium preceding the annual meeting of the American College of Obstetricians and Gynecologists

San Francisco, California
May 6, 1990

Chairman
Sebastian Faro, MD, PhD
Professor and Vice Chairman
Department of Obstetrics and Gynecology
Director of Section of Infectious Diseases
Baylor College of Medicine
Houston, Texas

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Groton, Connecticut

Chlamydia, the most common sexually transmitted disease, is currently treated with multiple-dose antibiotic regimens; however, azithromycin shows promise as single-dose therapy.

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Chlamydia trachomatis: Female pelvic infection

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Chlamydia trachomatis is a bacterium that causes both symptomatic and asymptomatic infection. This infection has been associated with severe infections of the upper genital tract, such as salpingitis, which may be responsible for a significant number of cases of ectopic pregnancy and infertility. An unsettled question is the role of *C. trachomatis* in pregnant patients. In addition to causing cervicitis, it has been linked to chorioamnionitis, premature rupture of membranes, and premature delivery. However, chlamydial infection is an easily treatable disease. The main focus of prevention is appropriate prescreening of patients at risk. (AM J OBSTET GYNECOL 1991;164:1767-70.)

Key words: Chlamydia, salpingitis, cervicitis, azithromycin

Chlamydia trachomatis is a unique bacterium in that it is a true parasite with an extracellular and intracellular phase of its life cycle. It has a specific requirement for adenosine triphosphate, which it must obtain from the host. Its ability to colonize a host and inflict significant tissue damage is thought to be responsible for a large number of cases of infertility and ectopic pregnancies. The spectrum of disease caused by *C. trachomatis* ranges from urethritis in both men and women to Bartholin's gland abscess, cervicitis, endometritis, salpingitis, neonatal conjunctivitis, and pneumonia.

Epidemiology

The actual incidence of *C. trachomatis* infection is not known. However, the best estimates of the frequency of isolation and recovery from patients with specific diagnosis rank it as the probable leading cause of sexually transmitted disease. It is difficult actually to appreciate the involvement of this organism in female pelvic infection because the infection is often asymptomatic.

Chlamydial infection has been liked to urethritis, proctitis, epididymitis, Bartholin's gland abscess, cervicitis, endometritis, salpingitis, perihepatitis, chorioamnionitis, premature rupture of membranes, and premature delivery.[1-11] This organism has also been linked, primarily through serologic association, to infertility and ectopic pregnancy.[12, 13]

The main problem with this particular infection is not in establishing a diagnosis but in educating physicians to assess each patient for risk factors (such as having more than one sexual partner), indicating that she should be screened for possible chlamydial infection. If they suspect that their partner is not monogamous, do they use oral contraceptive pills or birth control, are they single, have they been exposed to an individual with a sexually transmitted disease, or do they have a sexually transmitted disease? Several diagnostic tests are available, such as tissue culture, direct monofluorescent antibody for the detection of antigen (Mycotrak), modification of the enzyme-linked immunosorbent assay technique (Chlamydiazyme), and a rapid test called the TestPack (Abbott Laboratories, Chicago, Ill.).

It has been estimated that 20% to 40% of all sexually active women have been exposed to *C. trachomatis* and have positive antibodies to this organism.[14] The cervical colonization or infection rate in nonpregnant women has been estimated at 3% to 5%, whereas in pregnant patients it ranges from 3% to 25%.[15, 16] The isolation rates depend on the population studied; if one is studying a population at a sexually transmitted disease clinic, the prevalence will be higher than that in a private practice setting of middle to upper income patients. For example, in Houston, Texas, among sexually active adolescents (ages 13 to 19 years old), the prevalence of *Neisseria gonorrhoea* was 7.1% and 27.4% for *C. trachomatis*, whereas among patients admitted for pelvic inflammatory disease, the prevalence of gonorrhoea

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Table I. Infections caused by *C. trachomatis*

Serovar	*Infection*
A, B, Ba, C	Hyperendemic blinding trachoma
L1, L2, L3	Lymphogranuloma venereum
D, E, F, G H, I, J, K	Inclusion conjunctivitis, urethritis cervicitis, endometritis, salpingitis, epididymitis, and neonatal pneumonia

ranges from 10% to 81% and from 5% to 20% for cervical chlamydial infection.[12, 17, 18]

Infants born to women with chlamydial cervical colonization have a 60% to 70% risk of becoming infected during vaginal delivery.[19] It is estimated that approximately 25% to 50% of infants born to women with cervical colonization will develop conjunctivitis, and approximately 10% to 20% will develop pneumonia.[19-22]

Approximately 2 million cases of cervicitis and pelvic inflammatory disease are diagnosed annually.[22] About 400,000 cases are attributed to *C. trachomatis*, and these generate approximately 1 million ambulatory visits, 100,000 hospitalizations, 8050 cases of infertility, 13,900 ectopic pregnancies, and 280 deaths annually.[22] Thus the total annual cost, including indirect costs such as time lost from work, may exceed 2 billion dollars.[23, 24]

Pathophysiology

C. trachomatis is a true bacterium, because it has deoxyribonucleic acid, ribonucleic acid, and a cell wall that resembles that of gram-negative bacteria and is susceptible to antibiotics. *C. trachomatis* is an obligate intracellular parasite that has specific requirements for adenosine triphosphate and amino acids. Elementary bodies, the infectious form of the agent, attach to host cells by nonspecific forces and by binding to specific receptors.[25, 26] Endocytosis of the infectious particle occurs by a local segmental response of the host cell membrane, termed "parasite specified uptake."[27] Within the phagosome, the bacterium differentiates into the larger reticulate body form (also known as the initial form). A factor produced by the chlamydia inhibits fusion of the phagosome with cellular lysosomes, thus preventing destruction of the intracellular parasite. The specific mechanisms are unknown, but one hypothesis is that *Chlamydia* alters the protein composition of the endosomal membrane and thus prevents fusion with the lysosomes.[28] The reticulate body subsequently reproduces by binary fission. When the host cell is depleted of its adenosine triphosphate and essential amino acids, the newly formed reticulate bodies undergo a second metamorphosis and return to the elementary body form, thus completing the life cycle. The host cell is lysed and elementary bodies are released, allowing the chlamydia to infect new cells. The exact mechanism causing lysis of the host cell is unknown. However, studies have demonstrated that lysosomal enzymes are present in the cytoplasms of host cells.[29] The life cycle is completed within approximately 48 to 72 hours after entry into the host cell.

Table II. Infections caused by *C. trachomatis*

- Female
  - Bartholinitis
  - Urethritis
  - Cervicitis
  - Salpingitis
  - Preterm labor
  - Premature rupture of membranes
  - Chorioamnionitis
  - Postpartum endometritis
- Male
  - Epididymitis
  - Proctitis
  - Urethritis
- Neonate
  - Conjunctivitis
  - Pneumonia

Infection

Antigenic determinates define 15 serovars that cause three major types of infection (Table I). Infection is acquired by sexual intercourse or by contact of the mucous membrane with an infected site. The unique characteristics of *C. trachomatis* infection are its nondiscriminatory selection of the host (women, men, and children), its ability to cause asymptomatic but significant infection involving both the lower and upper genital tracts, and its potential for causing morbidity in the obstetric patient. Apparently the bacterium does not infect squamous epithelium but has a preference for columnar epithelium. The cervix is a prime target of the organism, although various other areas containing columnar epithelium are also capable of becoming infected (Table II).

Prevention

The best defense against this organism is a good offense. Condoms can be expected to reduce significantly transmission of this disease. It is imperative that the physician be alert to recognize the patient at risk. It would be neither prudent nor economically feasible to screen every patient seen for examination. If the patient is single and known to be sexually active, however, she should be screened by one of the rapid methods or culture techniques. The presumed risk factors are listed in Table III.

Individuals with a sexually transmitted disease, such as trichomonas, herpes, human papilloma virus, syphilis, and particularly, gonorrhea, should be thoroughly evaluated for *C. trachomatis*, because persons harboring one sexually transmitted disease are frequently infected with another. *N. gonorrhoea* and *C. trachomatis* are found coinfecting an individual in approximately 60% of the cases. Whenever possible, it is most appropriate to try to document the presence of chlamydial coinfection. Diagnosis is desirable to: (1) determine that treatment was effective when reexamining the patient, (2) provide specific information to the patient, (3) permit appropriate treatment of the sexual partner, and (4) determine the incidence of the infection within a given practice, which can provide information vital to the community.

Because *C. trachomatis* has been implicated in premature rupture of membranes, premature labor, chorioamnionitis, and postpartum endometritis, patients with any of these problems should be evaluated for the presence of this bacterium. However, current data have not proved conclusively a cause-and-effect relationship.[30, 31] Until that question is settled, if facilities are available to detect chlamydia, its presence or absence should be determined.

The problem with attributing postpartum endometritis to *C. trachomatis* is that no large studies have demonstrated a specific benefit from treating women with a positive culture for *C. trachomatis*. In fact several reports have documented the presence of this organism in patients with postpartum endometritis treated successfully with an antimicrobial agent that is not effective against chlamydia. The concern is that these patients are at risk for becoming infertile or will experience tubal damage and therefore will be at risk for ectopic pregnancy. Again, this has not been established by conclusive data.

On examination of the possibility of a relationship between chlamydial infection and infertility, it becomes immediately obvious that this hypothesis is based largely on immunologic data.[32, 33] Many patients who are infertile have positive serum antibodies (mainly immunoglobulin G) to *Chlamydia*. However, these data do not reveal whether these individuals experienced tubal damage because of chlamydial infection.

What is the incidence and range of chlamydial antibody among the general population? What is the incidence among persons known to have had cervical infection who subsequently become pregnant? It may be appropriate to conclude that because an individual has a positive immunoglobulin G antibody specific for *Chlamydia*, this is indeed the reason for infertility or ectopic pregnancy.

Conclusion

There is little doubt that *C. trachomatis* is one of the more frequently sexually transmitted diseases. This bacterium is commonly found to be responsible for urethritis and cervicitis. It has also been found in association with bartholinitis, endometritis, irregular uterine bleeding, and salpingitis. The frequency, however, remains to be determined. The role of this organism in obstetric patients with chorioamnionitis, premature rupture of membranes, and premature labor must still be elucidated.

Table III. Ten risk factors for acquiring *C. trachomatis*

Unmarried and sexually active
Multiple sexual partners
Use of oral contraceptive pills
Use of an intrauterine contraceptive device
History of a previous sexually transmitted disease
Known exposure with a partner having a sexually transmitted disease
Presence of a sexually transmitted disease organism, especially *N. gonorrhoeae*
Spotting after intercourse
Intermenstrual spotting whether or not the patient is taking oral contraceptive pills
Vague complaints of lower abdominal pain.

However, until the precise role of this bacterium is discovered, its presence or absence should be determined when a patient has one of these conditions. This should be done to perform a complete evaluation, determine the possible cause of the presenting condition, and administer appropriate treatment. The bacterium can be treated with a 7- to 10-day course of tetracycline, doxycycline, erythromycin, or clindamycin. Multiple-dose regimens under investigation include β-lactams, clindamycin-gentamycin combinations, and some of the fluoroquinolones. The fluoroquinolones and tetracyclines should not be used in pregnant or breast-feeding patients.

Single-dose therapy for cervicitis and urethritis caused by *C. trachomatis* is expected to eliminate the compliance problems associated with multiple-dose, multiple-day regimens. Index patients and their sexual partners could be easily treated, thereby eliminating disease recurrences resulting from noncompliance and reinfection.

The articles to follow in these symposium proceedings will present data on azithromycin, the first of the azalide class of antibiotics. The molecular characteristics of this drug result in extensive penetration of tissue and the attainment of high concentrations within cells.[34] Azithromycin's long half-life in tissue[35] is especially well matched to the prolonged life cycle of chlamydia. A single oral dose of 1 gm provides adequate levels of drug in infected tissues for a duration sufficient to eradicate infection. Azithromycin also demonstrates good in vitro activity against *N. gonorrheae* and some Enterobacteriaceae and anerobes.[36] It is excellent for the treatment of chlamydial urethritis and cervicitis.[37, 38] Azithromycin is likely also to contribute to the care of patients with pelvic inflammatory disease.

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Genital chlamydial infections: Epidemiology and reproductive sequelae

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Genital chlamydial infection is increasing and is now more common than gonorrhea. A sizable percentage of chlamydial infections of the lower genital tract in women progress to endometritis and salpingitis. Tubal infertility and ectopic pregnancy are important sequelae. Failure to control chlamydial infections reflects the following four factors: (1) Many cases are mild or asymptomatic; (2) diagnostic tests are expensive and technically demanding; (3) at least 7 days of multiple-dose therapy are currently required; and (4) partner notification is not routinely performed. Thus early identification of infected persons and compliance with curative therapy are less likely than with other sexually transmitted bacterial diseases. (AM J OBSTET GYNECOL 1991;164:1771-81.)

Key words: Pelvic inflammatory disease, endometritis, salpingitis, ectopic pregnancy, nonspecific urethritis

During the past two decades, genital chlamydial infections have surpassed gonorrhea as the number one sexually transmitted bacterial infection in the United States.[1] About 4 million chlamydial infections occur each year at an estimated cost of $2.2 billion for 1990.[2] As with gonorrhea, the most important implications of genital chlamydia are the reproductive health sequelae of upper genital tract infections in women. In addition, chlamydia causes genital tract complications in men. Chlamydia apparently produces less severe symptoms than gonorrhea, but it generally seems to smolder with a minimum of patient awareness until secondary or tertiary problems arise.[3, 4]

This review describes the epidemiology and reproductive sequelae of genital chlamydial infections and will concentrate on pelvic inflammatory disease (PID) and the complications of tubal infertility and ectopic pregnancy. The article that follows will elaborate on the effects of chlamydia on pregnancy.[5]

Epidemiology

Although genital chlamydial infection is not reportable in all 50 states, national trends can be estimated through physician surveys and sentinel surveillance projects. Using the syndrome of nonspecific urethritis (NSU) in men as a proxy for chlamydial infections, incidence levels have remained high in the 1970s and 1980s. In the United States, the number of visits to private physicians' offices for the diagnosis of NSU surpassed gonorrhea in 1972 (Fig. 1). The gap has widened in recent years, with NSU now twice as common as gonococcal urethritis in men. This elevated level of NSU is more remarkable when juxtaposed against the pattern of decreasing visits for all conditions to private physicians. Thus the percentage of NSU visits has actually been increasing.

A similar picture is found in Great Britain.[6] The British use the term nongonococcal urethritis (NGU) to estimate chlamydial infections. During the 1970s and 1980s, the incidence of NGU reported to British authorities more than doubled. In England and Wales, NGU now accounts for nearly three times as many infections in men as gonorrhea.

Another estimate of the annual incidence of genital chlamydial infections was derived from a model that used a ratio of chlamydial to gonococcal infections.[7] The ratio was based on data provided by sentinel sites during the mid-1980s and on a literature review of selected prevalence studies. In 1986 chlamydia was estimated to cause more than 4 million infections in the United States—approximately 2.6 million infections occurred in women, 1.8 million in men, and 250,000 in infants. The ratio of *Chlamydia trachomatis* to *Neisseria gonorrhoeae* infections was influenced by at least five variables besides age and sex. These included race, pregnancy status, choice of contraception, the proportion of infection without symptoms, and sexual preference. Considerably higher chlamydia to gonorrhea ratios were found among whites, pregnant women, oral contraceptive users, and persons without symptoms; lower ratios were found among homosexual men.

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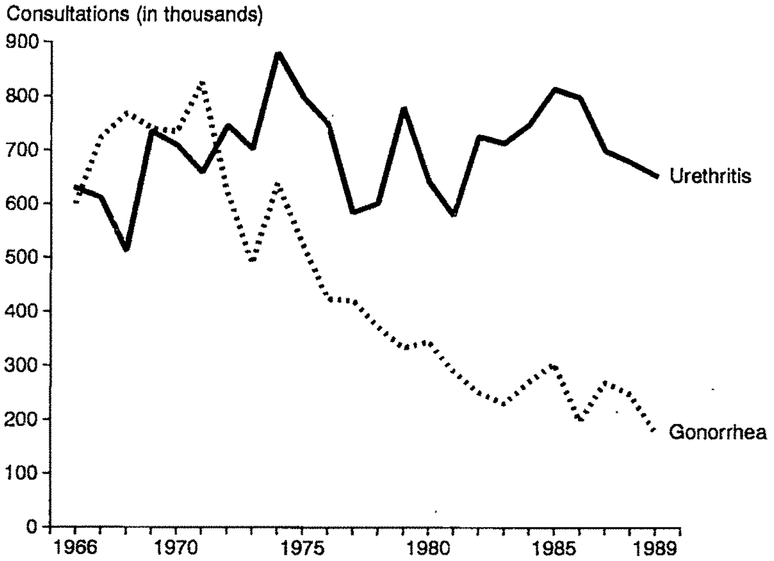

Fig. 1. Total consultations for nonspecific urethritis and gonorrhea among men in the United States from 1966 to 1989. Source: National Disease and Therapeutic Index (IMS America).

Data from many North American metropolitan areas have also documented the predominance of chlamydial compared with gonococcal infections. For example, in Seattle, a provider-based survey of urethritis in the 1970s found markedly higher rates for NSU than for gonococcal infection.[8] The Seattle NSU to gonorrhea ratio was higher in men seen by private practitioners than in those treated in health department clinics. In Denver the incidence of NGU rose rapidly in the late 1970s and 1980s, and it became the most prevalent sexually transmitted disease in this area by the early 1980s.[9]

Chlamydia also is prevalent outside of urban areas. In a rural province in Southern Quebec (Canada), prospective surveillance of genital infections found that *C. trachomatis* was three times more frequent than *N. gonorrhoeae.*[10] In Colorado Springs, Col., similar distributions occurred.[11]

Chlamydia is apparently more difficult to transmit than gonorrhea.[12] Based on extrapolations from partner notification programs, within couples with discordant infection status, men will transmit *C. trachomatis* to their female partners 40% of the time, whereas women will transmit the infection to their male partner 32% of the time. Because these infection risks are based on the average frequency of intercourse for each couple, the transmission probability from a single coital episode is probably much lower.

During the past 5 years, a plethora of articles have been published on the prevalence and characteristics of chlamydial infections in women. These have sharpened the epidemiologic focus regarding the magnitude of infection and the consistency of risk factors. Depending on the population of women screened, the prevalence of genital chlamydia appears to range from 8% to 40%, with a typical median of 15%.[13-15] Demographic risk markers for chlamydia have been remarkably uniform—young age, unmarried status, and lower socioeconomic conditions predict higher rates. Other risk factors for cervical chlamydia in women are anatomic (ectopy), behavioral (number of sexual partners), microbiologic (concurrent gonorrhea), and hormonal (use of oral contraception).

Because of the consistency of these risk markers and factors and the financial and technical constraints on routine testing for *C. trachomatis*, attempts have been made to generate a sensitive set of predictors of chlamydial infection on the basis of nonlaboratory data from patient histories or physical examinations. For example, among patients attending a sexually transmitted disease clinic in Denver, multiple logistic regression was used to define sets of risk markers and factors that would identify patients with chlamydial infections.[16] In heterosexual men with urethral discharge, among whom 27% were infected with *C. trachomatis*, Gram's stain evidence of nongonococcal urethritis, young age, history of urethral discharge for more than a week, and absence of dysuria were factors that identified subgroups with a prevalence as high as 64%. In women, among whom the overall prevalence of infection was 17%, young age, unmarried status or a contact with gonorrhea, presence of cervical erythema or mucopus, evidence of PID, and absence of recent antibiotic use identified subgroups with a prevalence up to 61%. On the basis of these data, the investigators suggested a strategy of empiric therapy for women with four or more of these factors, laboratory testing for women with two or three of the factors, and neither testing nor

Table I. Detection of *C. trachomatis* and *N. gonorrhoeae* among women with acute PID: Selected studies

	C. trachomatis			*N. gonorrhoeae*	
Study (yr)	*Cervix*	*Tubes*	*Antibody*	*Cervix*	*Tubes*
Europe					
Adler et al.[50] (1982)	5%	—	31%	18%	—
Moller et al.[45] (1981)	22%	—	20%	5%	—
Eilard et al.[58] (1976)	27%	9%	—	32%	5%
Paavonen et al.[53-55] (1979, 1980, 1981)	32%	—	26%	26%	—
Ripa et al.[51] (1980)	33%	—	46%	19%	—
Mardh et al.[47, 48] (1977, 1981)	38%	30%	37%	17%	7%
Henry-Suchet et al.[45] (1980)	38%	24%	—	0%	25%
Moss et al.[56] (1986)	41%	—	—	9%	—
Stacey et al.[57] (1987)	44%	—	—	27%	—
Gjonnaess et al.[49] (1982)	46%	16%	40%	8%	0%
Osser et al.[52] (1982)	47%	—	51%	20%	—
North America					
Sweet et al.[61] (1981)	5%	0%	23%	46%	23%
Hemsell et al.[65] (1988)	7%	—	—	56%*	—
Thompson et al.[62] (1980)	10%	10%	—	80%	33%
Brunham et al.[66] (1988)	14%	8%	40%	42%	18%
Eschenbach et al.[60] (1975)	20%	2%	20%	44%	13%
Sweet et al.[63, 64] (1977, 1980)	27%	—	—	40%	—
Cromer et al.[67] (1987)	39%	—	—	27%	—
Bowie et al.[59] (1981)	51%	—	—	35%	—

Modified from Cates W Jr, Rolfs RT, Aral SO. Sexually transmitted diseases, pelvic inflammatory disease, and infertility: an epidemiologic update. Epidemiol Rev 1990;12:199-220, by permission.

*Endometrial specimens.

treatment for the remainder. In their population this would result in antibiotic coverage of 98% of infected women while testing only 68% of clinic patients and unnecessarily treating only 9%.

Such analyses are useful primarily for local purposes to determine the actual yield of particular screening approaches. Other recent attempts to design such algorithms[17-21] have varied depending on the population to which they are applied. Thus the use of specific demographic markers, medical histories, and physical examination results to exclude persons from screening must be carefully tailored to the type of facility, characteristics of the patients, and availability of resources.

Natural history

The natural history of the chlamydial infections of the lower genital tract is uncertain. Many chlamydial infections are asymptomatic or not easily recognized on clinical grounds alone. Asymptomatic carriage in both men and women may be prolonged, further increasing the potential for transmission.[22] As many as 25% of men identified with urethral infections caused by *C. trachomatis* have no signs or symptoms of infection.[23, 24] These asymptomatic men may be an important source of infection to women unless identified through screening tests and appropriately treated.[25]

As many as 70% of women with cervical infection caused by *C. trachomatis* may be essentially without symptoms,[26] although mild symptoms of vaginal discharge, bleeding, lower abdominal pain, or dysuria may be present.[26, 27] Asymptomatic infection of the urethra and rectum caused by *C. trachomatis* can accompany infection of the cervix.[28, 29] In some women, *C. trachomatis* infection of the urethra results in symptomatic urethritis with dysuria and pyuria.[30]

Furthermore, asymptomatic chlamydial infection may lead to epidemiologic patterns that differ substantially from those of gonococcal infection. A recent study in Baltimore characterized chlamydial infections as "prevalent" infections because, in contrast to the "incident" pattern of gonococcal disease, chlamydia rates did not vary significantly by reason for clinic visit, number of sexual partners, or classification of partners as new, regular, or casual.[31]

The percentage of untreated chlamydia of the lower genital tract that ascends may be quite high. One study estimated that approximately 8% of untreated chlamydial infections progress to salpingitis[32], however, among women treated for gonorrhea with penicillin whose subsequent culture showed simultaneous cervical chlamydia, 40% had developed signs of upper genital tract infection during the 7-day follow-up interval.[33] Other studies have shown that cervical chlamydia treated only with β-lactam antibiotics tends to hibernate in the endometrium.[34] Finally, douching may play a role in producing upper genital tract infection in women with chlamydia of the lower genital tract.[35]

Oral contraceptives appear to promote acquisition of chlamydial infection of the lower genital tract.[36, 37] For example, in a recent randomized prospective study that controlled for differences in demographic factors, sexual behavior, and cervical ectopy, oral contraceptive

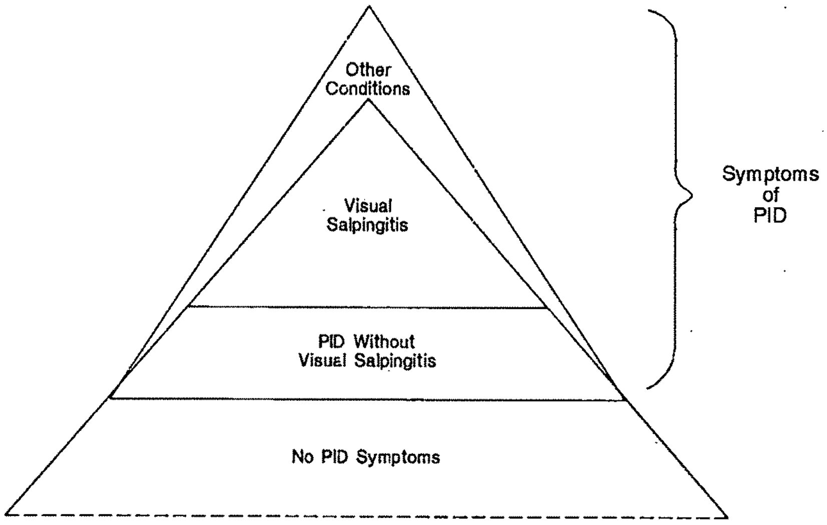

Fig. 2. Schematic representation of symptomatic and asymptomatic PID.

users developed 70% more chlamydial and gonococcal infections than women relying on intrauterine devices and tubal ligation.[38] Cervical ectopy appeared partially to explain the increased risk of chlamydial infection but did not contribute to the higher gonorrhea rates. A study in guinea pigs inoculated intravaginally supports these human data.[39] Oral contraceptive–treated guinea pigs were more likely than control animals to have prolonged chlamydial infection of the lower tract, with higher numbers of organisms and delayed appearance of local antibodies.

Whether the increase in cervical chlamydial infection associated with oral contraception affects the risk of endometrial or tubal infection is unclear.[36] In Seattle, women with cervical chlamydia who used oral contraception were less likely to develop symptoms of upper genital tract infection than those using no contraceptives.[40] Whether oral contraceptives truly reduced tubal infection or possibly suppressed inflammatory symptoms could not be resolved by this study. In addition, these data are inconsistent with results from animal models[41] and analytic studies of tubal factor infertility.[42]

Finally, chlamydial cervicitis may alter the natural history of cervical atypia. Recent preliminary data from Finland indicate that women with persistent koilocytotic atypia were almost three times as likely to have chlamydial infection (alone or together with gonorrhea) as were women in whom koilocytosis resolved. Although the association did not reach statistical significance, it is consistent with other data linking chlamydial infections to an increased risk of cervical neoplasia.[43]

Sequelae: PID

Both microbiologic and serologic measures have established *C. trachomatis* as an important sexually transmitted organism leading to upper genital tract infection.[44] However, the rate at which chlamydial and gonococcal organisms have been recovered in patients with symptoms of PID varies widely. This variation is probably from differences among the populations studied, in the time period during which the investigations took place, in methods used to recover the organisms, and in the severity of infection.

In both Europe and North America, more recent investigations[45-67] have generally found a higher proportion of *C. trachomatis* than *N. gonorrhoeae* in women with symptoms of PID (Table I). In Scandinavia, between one quarter and one half of PID was associated with culture, serologic evidence, or both of *C. trachomatis* infection. Chlamydia has also been isolated in as many as 51% of some North American populations.[59]

In nearly all studies of women with symptoms of PID in whom abdominal cultures were taken, chlamydia was isolated at a lower rate from tubal than from cervical specimens (Table I). This is not unexpected, since extraluminal swabs may not be as efficient in obtaining organisms as endocervical techniques and smaller numbers of organisms may reach the tubes than reach the cervix. Moreover, local neutralizing antibodies may limit culture sensitivity at the intratubal level. Detection of chlamydia antibodies, indicative of either present or past infection, was usually more frequent than isolation of organisms from the cervix or tubes.

Based on our evolving knowledge of PID, asymptomatic ("silent") or "atypical" syndromes have assumed a greater importance (Fig. 2). Because of its smoldering nature, chlamydial infection plays a key role in this problem. Chronic, subacute, or latent endometrial and tubal infection is probably present in a large number of women, but a consensus definition of these clinically subtle infections is lacking.[68] Moreover, the magnitude of the problem is unknown. Endometritis has been

Table II. Case-control studies of previous *C. trachomatis* infection and subsequent tubal infertility

Study/location (yr)	*C. trachomatis antibody level*	*Study populations*	*Findings of antibody positive/tubal occlusion (%)*
Punnonen et al.,[75] Turku, Finland (1979)	MIF ≥1:16	Infertile women with abnormal HSG	91
		Infertile women with normal HSG	51
Henry-Suchet et al.,[45] Paris, France (1980)	MIF ≥:64	Infertile women with tubal obstruction	26
		Infertile women without tubal obstruction	4
Ballard et al.,[76] Johannesburg, South Africa (1981)	MIF ≥1:8	Infertile women with tubal disease	87
		Pregnant women	38
Jones et al.,[77] Indianapolis, Ind. (1982)	MIF ≥1:8	Infertile women with chlamydia antibody	75
		Infertile women without chlamydia antibody	28
Moore et al.[78] Seattle, Wash. (1982)	MIF ≥1:32	Infertile women with tubal occlusion	75
		With adhesions	21
		Infertile women without tubal conditions	0
Gump et al.,[79] Burlington, Vt. (1983)	MIF ≥1:16	Infertile women with abnormal HSG	64
		Infertile women with normal HSG	28
Cevenini et al.,[80] Bologna, Italy (1982)	MIF ≥1:8	Infertile women with salpingitis	90
		Infertile women without salpingitis	27
Padjen et al.,[81] Leyden, The Netherlands (1984)	EIA	Infertile women with inflammatory tubal abnormalities	83
		Infertile women without tubal abnormalities	16
Conway et al.,[82] Bristol, England (1984)	MIF ≥1:32	Infertile women with tubal damage	75
		Infertile women without tubal damage	31
Kane et al.,[83] London, England	MIF ≥1:8	Infertile women with hydrosalpinx	41
		Infertile women with normal tubes	12
Battin et al.,[84] Los Angeles, Calif. (1984)	MIF ≥1:32	Infertile women with tubal disease	94
		Infertile women without tubal disease	86
Brunham et al.,[85] Winnipeg, Canada (1985)	MIF ≥1:32	Infertile women with tubal damage	72
		Infertile women without tubal damage	9
Moller et al.,[86] Aarhus, Denmark (1985)	MIF ≥1:32	Infertile women with abnormal HSG	33
		Infertile women with normal HSG	13
Kelver et al.,[87] Galveston, Texas (1989)	MIF ≥1:16	Infertile women with positive chlamydia serology	77
		Infertile women with negative chlamydia serology	35
Hawes et al.,[88] Melbourne, Australia (1986)	MIF ≥1:160	Infertile women with tubal damage	67
		Infertile women for other reasons	7
		Pregnant women	10
Guderian et al.,[89] Los Gatos, Calif. (1986)	MIF ≥1:32	Infertile women with tubal disease	83
		Infertile women with normal tubes	16
Quinn et al.,[90] Toronto, Canada (1987)	MIF ≥1:32	Infertile women with tubal occlusion	71
		Infertile women with ovulation defects	19
		Normal women	16
Anestad et al.,[91] Oslo, Norway (1987)	MIF ≥1:8	Infertile women with tubal occlusion	91
		Infertile women with normal tubes	55
		Pregnant women	47
Reniers et al.,[92] Franceville, Gabon (1989)	MIF ≥1:64	Infertile women with tubal obstruction	N/A
		Infertile women with normal tubes	–
		Pregnant women	–
Sellors et al.,[93] Hamilton, Canada (1988)	EIA	Infertile women undergoing tuboplasty	81
		Women with tubal ligation	37
		Women having hysterectomy	33
Minassian et al.,[94] Philadelphia, Pa. (1990)	EIA	Infertile women with chlamydia antibody	87
		Infertile women without chlamydia antibody	14

MIF, Microimmunofluorescence; *HSG,* hysterosalpingogram; *EIA,* enzyme immunoassay; *NA,* not available.

Table III. Studies comparing both chlamydial and gonococcal antibodies associated with tubal infertility

Study/location (yr)	*Study populations*	*Chlamydial antibodies*		*Gonocccal antibodies*	
		%	*Relative risk*	%	*Relative risk*
Mabey et al.,[95] The Gambia (1985)	Tubal infertility	68	1.9 (1.2-3.1)	68	2.4 (1.4-4.1)
	Pregnant women	35	1.0	27	1.0
Tjiam et al.,[96] The Netherlands (1985)	Tubal infertility	21	α	61	
	Infertile–normal tubes	0	1.0	25	$p < 0.1$
	Blood donors	0	1.0	4	1.0
Robertson et al.,[97] Bristol, England (1987)	Tubal infertility	73	1.9 (1.3-2.9)	2	—
	Infertile–normal tubes	34	0.9 (0.5-1.5)	3	—
	Barrier contraceptives	25	0.7 (0.4-1.2)	4	NS
	Sterilization	38	1.0	5	—
Miettinen et al.,[98] Tampere, Finland (1990)	Tubal infertility	46	11.1 (3.1-40.3)	14	—
	Infertile–normal tubes	7	1.0	0	1.0
WHO Multicenter[99] (unpublished) (1990)	Tubal infertility	55	3.9 (2.1-7.3)	44	3.7 (1.3-10.5)
	Infertile–normal tubes	30	1.4 (0.8-2.3)	17	1.0 (0.3-2.6)
	Pregnant women	24	1.0	18	1.0
DeMuylder et al.,[100] Gweru, Zimbabwe (1990)	Tubal infertility	39	8.7 (3.6-22.2)	52	20.7 (7.5-61.7)
	Infertile–normal tubes	5	0.9 (0.4-3.3)	25	8.3 (2.6-29.6)
	Pregnant women	7	1.0	5	1.0

WHO, World Health Organization; *NS,* not significant.

found in 40% of women with cervicitis who had no symptoms of PID at the time of referral.[69] Conversely, in three of nine women with symptoms of PID but normal external tubal serosa at laparoscopy, endometrial biopsy specimens revealed plasma cell endometritis; moreover, several weeks later the endometrial infection eventually led to salpingitis.[70]

Thus PID, which both causes symptoms and also produces visually detectable salpingitis, probably accounts for less than half of the total cases. After a decade of using the term "acute salpingitis" to provide specific diagnoses, clinical evidence now appears to favor a more sensitive definition of PID. Not only does the upper genital tract infection affect more than just the fallopian tubes but also the tubes themselves can be involved without visual evidence of salpingitis. Future efforts to identify women with smoldering upper genital tract infection will have to rely on atypical symptoms (e.g., abnormal or increased bleeding) or biochemical markers (e.g., tumor-associated trypsin inhibitor and C-reactive protein).

Sequelae: Tubal infertility

Acute salpingitis, whether related to *C. trachomatis, N. gonorrhoeae,* both organisms, or neither, is causally related to tubal infertility.[71] However, silent salpingitis accounts for a sizable proportion of tubal infertility, probably the majority. Nearly all investigations have found that more than half of the women with documented tubal occlusion report no history of previous PID despite serologic evidence of past chlamydial infection.[72, 73] Moreover, morphologic and physical analyses of tubal epithelium from women with distal tubal obstruction found extensive ultrastructural damage, even in those without knowledge of previous PID.[74]

A large number of investigations from 14 countries (Table II) have examined the relationship between serologic evidence of past chlamydial infection and tubal infertility.[74-94] Despite wide variations in design, these studies have uniformly found a significant association between tubal occlusion and serologic evidence of prior chlamydial infection. In addition, five of the six additional studies,[95-100] which included both chlamydial and gonococcal antibodies (Table III), implied independent etiologic associations between the infections and tubal infertility. Only one study in Bristol, England, found no relation to previous gonococcal infection.

Cohort studies verify the link between sexually transmitted diseases, PID, and tubal infertility. In Lund, Sweden, among women with symptoms of laparoscopically verified salpingitis, similar levels of tubal occlusion occurred regardless of whether gonorrhea, chlamydia, both organisms, or neither was isolated from the cervix.[101, 102] In Winnipeg further evidence of PID sequelae was confirmed in a study of 50 women with laparoscopically verified acute salpingitis.[66] Of the 50 women, 23 attempted to become pregnant and had a subsequent evaluation to determine their fertility status. Seven of 13 women with nongonococcal infection had an adverse reproductive outcome compared

Table IV. Case-control studies of previous *C. trachomatis* infections and subsequent ectopic pregnancy

Study/location (yr)	*Population*	*Chlamydia antibody (%)*	*Relative risk crude/adjusted*
Svenson et al.,[113]	112 Women with EP	56	3.1
Lund, Sweden (1985)	56 Postsalpingitis	84	
	56 Other	44	
	86 Pregnant women	21	1.0
Brunham et al.,[111]	50 Women with EP	—	
Winnipeg, Canada (1986)	36 Without risk factors	56	2.5
	14 With IUD/TL	18	—
	49 Pregnant women	22	1.0
Hartford et al.,[115]	24 Women with EP	—	α
Los Angeles, Calif. (1987)	10 Tubal disease	50	—
	14 No tubal disease	0	—
Robertson et al.,[116]	50 Women with EP	32	7.9
Southampton, England (1988)	50 Pregnant women	4	1.0
Walters et al.,[117]	60 Women with EP	66	6.6 (adjusted)
San Antonio, Texas (1988)	60 Pregnant women	20	1.0
Chaim et al.,[118]	35 Women with EP	32	4.9
Beer Sheva, Israel (1989)	294 Healthy women	8	1.0
Miettinen et al.,[98]	20 Infertile women with EP history	40	5.7
Tampere, Finland (1990)	28 Infertile women with normal tubes	7	1.0
Chow et al.,[119]	306 Women with EP	71	2.4 (adjusted)
Los Angeles, Calif. (1990)	266 Pregnant women	39	1.0
Sherman et al.,[122]	135 Women with EP	—	2.1 (adjusted)
Seattle, Wash. (1990)	192 Random women in prepaid health plan	—	1.0
DeMuylder et al.,[130]	60 Women with EP		
Gweru, Zimbabwe (1990)	39 Abnormal tubes	26	4.8
	21 Normal tubes	5	0.9
	104 Pregnant women	7	1.0

EP, Ectopic pregnancy; *UD/TL*, intrauterine device/tubal ligation.

with none of 10 women with gonococcal infection ($p = 0.007$). Two factors were associated with adverse reproductive outcome: four of the seven infertile women had initial tubal abscesses, and three had evidence of *C. trachomatis.*

Chlamydia apparently causes more severe subclinical tubal inflammation and, ultimately, tubal damage than other agents despite its more benign-appearing symptoms and signs.[103-105] Colonization of the fallopian tube by *C. trachomatis* has been found in infertile women without symptoms in the absence of laparoscopic signs of active pelvic infection.[106] The severity of tubal damage despite mild clinical symptoms is probably because chlamydial salpingitis is more chronic than salpingitis caused by other organisms. This hypothesis is supported by the low prevalence of immunoglobulin M antibody to chlamydia among women with acute salpingitis.[18]

The mechanism that accounts for tubal occlusion after chlamydial infection is unknown. An indirect and long-term process may be responsible. Animal studies have demonstrated that single chlamydial infections have been self-limiting with little residual damage; however, repeated chlamydial infection eventually produces tubal occlusion and infertility.[107] Serial inoculations of chlamydia directly into the fallopian tube of pig-tailed monkeys result in intratubal adhesions and distal tubal obstruction.[108] Similar histopathologic findings occurred after tubal challenge that followed repeated chlamydial cervical infection.[109] Whether this represents immune-mediated tubal destruction or exacerbation of chronic, active chlamydial infection is not clear.[110] γ-Interferon may also be involved with chlamydial mechanisms of pathogenesis.[111] Untreated chlamydial salpingitis may show symptoms of resolution in the face of progressive damage. Moreover, in mice, treatment with tetracycline had to be started early in the course of tubal infection to have any influence in reducing infertility.[112]

Sequelae: Ectopic pregnancy

Ectopic pregnancy is also associated with prior chlamydial PID. Several case-control studies[113-120] have demonstrated an association between chlamydial infection and ectopic pregnancy, with adjusted relative risks ranging between 2.4 and 7.9 (Table IV). The cohort study of salpingitis in Lund, Sweden, provides the strongest etiologic evidence of an association between PID and ectopic pregnancy.[121] Of 544 women with acute salpingitis proved at laparoscopy, 7% of the first pregnancies in these women were ectopic. Among control women with no acute salpingitis, less than 1% of the subsequent pregnancies were ectopic.[102]

Case-control studies of ectopic pregnancy that incorporated serologic tests for chlamydial infection have also shown strong associations (Table IV). The relationship between chlamydial serologic findings and ectopic pregnancy was particularly high among women who did not use intrauterine devices at conception. In England, chlamydial antibodies were more than two times more frequent than gonococcal antibodies among women with ectopic pregnancy.[116] However, evidence of previous infection with both these organisms was significantly higher in women with ectopic pregnancy than in the those with intrauterine pregnancies. In addition, an independent association between *C. trachomatis* and ectopic pregnancy was found in a recent study from the United States that controlled for douching as a risk behavior.[119]

Moreover, "silent" salpingitis may also be related to ectopic pregnancy.[122] Among 27 women who had an ectopic pregnancy after attending an infertility clinic, previous laparoscopy had indicated "normal" tubal morphologic findings in 12 (44%). Eight fallopian tubes were available from these 12 women for histologic examination after the ectopic pregnancy. All eight had evidence of ongoing, low-grade salpingitis. These findings suggest that laparoscopy cannot exclude smoldering intraluminal tubal disease in some women with normal serosal findings.

Conclusion

Genital chlamydial infections remain an important cause of preventable reproductive morbidity in the United States. During the past two decades, we have learned much about the factors related to infection with *C. trachomatis* and the insidious sequelae that eventually result. Failure to control chlamydial infections is a reflection of the following four factors: (1) Both symptoms and signs of chlamydial infection are often either extremely mild or totally absent; (2) tests for *C. trachomatis* are expensive and technically demanding; (3) at least 7 days of multiple-dose therapy are necessary to eradicate the organism; and (4) partner notification is not routinely performed for patients with chlamydial syndromes. Together these factors mean that early identification of infected individuals and compliance with curative therapy are less likely to occur with chlamydial infection than with other bacterial sexually transmitted diseases. However, when aggressive programs to control chlamydia are undertaken by communities, a measurable impact on lowering these smoldering genital infections can result.[123, 124]

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Chlamydia trachomatis infection during pregnancy

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Although transmission of *Chlamydia trachomatis* to infants during vaginal birth can result in conjunctivitis and pneumonitis, there is uncertainty about other adverse effects of chlamydial infection during pregnancy. There is some evidence that it may contribute to adverse complications such as premature rupture of membranes, preterm labor and birth, low birth weight, and still birth. Infection with *C. trachomatis* is also implicated in postabortal, postcesarean section, and postpartum maternal infections. Treatment of chlamydial infection during pregnancy has proved beneficial in the prevention of neonatal morbidity and is now recommended by the Centers for Disease Control. (Am J Obstet Gynecol 1991;164:1782-9.)

Key words: *C. trachomatis*, premature rupture of membranes, conjunctivitis, pneumonitis, neonatal morbidity

Chlamydia trachomatis is a well-established cause of pelvic inflammatory disease (endometritis or salpingitis) in women, urethritis and epididymitis in men, and conjunctivitis and pneumonitis in newborn infants. In the United States, an estimated 155,000 infants are exposed to the organism during the birth process each year, and more than 100,000 become infected.[1]

Interest in the possible roles of chlamydia in perinatal infection began more than 80 years ago with von Halberstaedter and Prowazek's[2] description of neonatal conjunctivitis associated with parental cervicitis, urethritis, and the microscopic presence of inclusion bodies. They first observed these organisms in conjunctival cells from patients with trachoma and named them "chlamydozoa," from the Greek word for "cloak." Even today uncertainty continues with regard to the following: (1) the role of chlamydia in maternal and neonatal infectious morbidity; (2) the extent to which chlamydia infection during pregnancy contributes to premature rupture of membranes (PROM), preterm birth, low birth weight, and puerperal infection; and (3) cost-effective approaches to the evaluation and treatment of pregnant women potentially infected with chlamydia.

We will briefly review the present understanding of this common sexually transmitted infection during pregnancy and offer recommendations for management. More comprehensive reviews of chlamydial infection during pregnancy are available.[3, 4]

Epidemiology

Epidemiologic aspects of chlamydial infection of the reproductive tract have been reviewed.[3, 5, 6] The prevalence of both symptomatic and asymptomatic genital chlamydial infection varies from under 2% among private obstetric patients to well over 20% among groups of younger women receiving care at public clinics. Epidemiologic factors associated with increased risk of infection include age less than 24 years, nonwhite race, use of oral contraceptives, and nonuse of barrier forms of contraception.[7-10]

Infection occurs most frequently among women aged 15 to 21 years and declines markedly with advancing age; in contrast, the prevalence of chlamydial antibody positivity increases with age until approximately age 30 years, when it plateaus in women at up to 50%.[11] Epidemiologic factors are useful to predict rates of infection among some groups of pregnant women but not to determine the presence of infection among persons.[12]

With rare exception, transmission from the infected mother to her newborn infant occurs during vaginal delivery through an infected cervix, and transmission rates range from 23% to 70%.[6, 13] Higher rates of vertical transmission are microbiologically documented if exposed infants have serial cultures done over several months.[14] Several instances of infection before birth or among infants born by cesarean section have also been reported.[15-17] Frommel et al.[8] noted an association with breast-feeding and suggested that intimate handling of newborn infants may play a role in transmission. Nevertheless, it appears that the vast majority of neonatal chlamydial infections occur during birth and thus are amenable to identification and preemptive treatment during pregnancy.

Microbiology and pathophysiology

C. trachomatis (genital serovars D to K) are small obligate, intracellular, gram-negative bacteria. Chlamydiae develop intracellularly within cytoplasmic vacuoles. Their unique developmental cycle includes production of precursor initial and reticulate bodies and release of infectious elementary bodies. Release of the

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elementary bodies occurs by cell rupture, with subsequent cell death accounting for direct tissue and organ damage. Such intracellular organisms are relatively protected from humorally mediated host defenses, which may account for the typically low antibody levels in uncomplicated infection and the prolonged persistence of untreated infection. Despite difficulties culturing *C. trachomatis*, elementary bodies are not fragile and may be infectious for several days.[5] Elementary bodies appear to induce phagocytosis by "nonprofessional" phagocytic cells, including squamocolumnar epithelia found in conjunctiva, the respiratory tract, and the mucous membranes of the genitourinary tract.

Approximately 30% to 40% of endocervical infections spread to the contiguous endometrium.[18] Infection and destruction of cervical, endometrial, and fallopian tube-lining cells may impair fertility, increase the risk of ectopic pregnancy, or damage a developing pregnancy. It is speculated that pregnancy loss, inadequate fetal development, bleeding, preterm labor, or PROM may result from persistent upper reproductive tract infection by *C. trachomatis*.

Other effects of chlamydial infection may also interfere with subsequent pregnancy. It is possible that presentation of host chlamydial epitopes on infected cell surfaces may induce altered immunologic responses. In an animal model of salpingitis, Patton et al.[19] demonstrated a florid inflammatory response to reinoculation of chlamydia. It is thought that an autoimmune reaction to chlamydial infection is the most common cause of Reiter syndrome or reactive arthritis among patients with permissive major histocompatibility (MHC) gene allotypes.[11]

Similarly, acute or persistent chlamydial infection of endometrial or decidual cells or fetal trophoblast cells may fundamentally alter maternal-fetal tolerance mechanisms that are integral to successful pregnancy. MHC molecules are normally suppressed in cells adjacent to the maternal-fetal interface. In vitro infection of decidual cells with *C. trachomatis*, however, sharply increases cell surface production of MHC molecules essential for immunologic recognition.

Infection with chlamydia also increases production and release of tumor necrosis factor, interferons, and interleukin-2 by infected cells.[20] Increased local tissue concentrations of these cytokines may enhance immunologic surveillance and decrease cell-mediated tolerance at maternal-fetus interfaces. Moreover, when inflammatory cells such as monocytes are exposed to *C. trachomatis*, they release relatively large amounts of interleukin-1. This cytokine may directly damage infected cells and is well known to amplify the response to other inflammatory mediators.

Endocrinologic changes associated with pregnancy may increase the virulence of both symptomatic and asymptomatic chlamydial infection. With the use of a

Rank et al.[21] demonstrated that treatment with estradiol resulted in florid endometritis, salpingitis, and cystitis. In contrast, infection in control animals was limited to the vagina and the cervix. Moreover, direct *C. trachomatis* infection may occur more commonly in trophoblastic tissue and the conceptus than is generally recognized. Harrison and Riggin[22] demonstrated that preparations of human amnion cells were as easily infected with chlamydia as were 5-iodo-2-deoxyuridine–treated cells used in routine culture. Neeper et al.[23] also demonstrated robust chlamydial infection of human amnion cells in fetal membrane explants. The failure of *C. trachomatis* to cause frequent intraamniotic infection may be explained by an as yet unidentified antichlamydial factor present in human amniotic fluid.[24] The possible importance of as yet unstudied aspects of chlamydial host susceptibility and strain virulence is suggested by several well-described instances of maternal sepsis caused by *Chlamydia psittaci* with intrauterine infection and fetal death.[4]

Neonatal transmission

Vertical transmission of *C. trachomatis* during vaginal birth typically occurs in 50% to 60% and as many as 70% of infants born to infected women.[12, 13, 25, 26] Prospective serial microbiologic surveillance has shown that colonization or infection may take weeks to months to become detectable[12] and that multiple sites (conjunctiva, nasopharynx, rectum, and vagina) may become infected or colonized.[12, 25, 26] Not all exposed or infected infants develop recognizable findings or symptoms; risks of clinically recognized conjunctivitis range from 18% to 50%.[25-29] Prospective examination of lacrimal fluid for inflammatory changes or microimmunofluorescent antichlamydial antibody testing may be a more sensitive measure of infection than cultures.[25] Recent studies show that 1% silver nitrate ($AgNO_3$) can be as effective as erythromycin or tetracycline ophthalmic ointments in prophylaxis of chlamydial conjunctivitis.[28] This suggests that prior estimates of vertical transmission that used newborn infants treated with silver nitrate as control subjects gave falsely low results.

Newborn chlamydial conjunctivitis occurs several weeks post partum and frequently runs a mild course ("glue eye"). However, severe exudative ophthalmitis can occur. On rare occasions, conjunctival scarring may be noted. It is commonly hypothesized that after chlamydiae have infected the conjunctiva, elementary bodies pass through the lacrimal duct to the nasopharynx and subsequently to the lower respiratory tract. Nasopharyngeal inoculation also occurs primarily at the time of birth; Hammerschlag et al.[27] found that the nasopharynx was the most common site of recovery after birth. Alexander and Harrison[29] noted that 15% to 20% of exposed infants developed nasopharyngeal infection or colonization and that a full one third de-

dence of chlamydial pneumonia was between 10% and 16%.[27, 29] Although most other investigators show similar results, prospective studies by Heggie et al.[13] do not show any increase in respiratory illness among infants exposed to chlamydia at birth.

Chlamydial pneumonitis is usually recognized between 6 weeks and 6 months of life.[30] Although chlamydial pneumonia is usually not progressive, affected patients frequently require hospitalization and may be predisposed to prolonged respiratory disease with impaired lung function. Chlamydial pneumonitis is clinically indistinguishable from pneumonitis caused by other pathogens infecting the newborn infant.[8, 30] As many as two thirds of cases of chlamydial pneumonitis may be associated with concomitant cytomegalovirus, *Ureaplasma urealyticum*, or *Pneumocystis carinii* infection.[8, 30] Both cytomegalovirus and genital mycoplasmas may also be transmitted to the fetus around the time of birth.

Approximately one half of the cases of pneumonitis occur in the absence of clinically recognized conjunctivitis.[29] The absence of prodromal conjunctivitis belies the assumption that chlamydial pneumonitis can be prevented by systemic treatment of newborns with conjunctivitis.

C. trachomatis is also implicated but not yet proved as a cause of otitis media, bronchiolitis, vulvitis, proctitis, and gastroenteritis in infants. Although neonatal conjunctivitis is usually not severe, topical treatment is often ineffective. Oral erythromycin is used in the newborn infant to treat chlamydial conjunctivitis effectively, as well as to reduce or eliminate chlamydial infection at other sites.

Postabortal and postpartum maternal infection

Women with cervical infection from *C. trachomatis* at the time of elective abortion are at increased risk for postabortal endometritis. In prospective European studies, 22% to 38% of chlamydia-infected women develop postabortal pelvic infection compared with 2% to 10% of those without chlamydia.[31, 32] Chlamydial postabortal endometritis tends to occur approximately 3 to 10 days later than nonchlamydial endometritis. It is not known whether endometritis, which is found in 30% to 40% of nonpregnant women with cervical infection,[18] affects risks of postabortal infection or whether chlamydia-infected women aborting spontaneously carry similar risks.

Optimally, women should be screened and treated for both *C. trachomatis* and *Neisseria gonorrhoeae* before termination of pregnancy. Alternatively, tests for *C. trachomatis* could be done immediately before the procedure, with treatment initiated promptly after the microbiologic diagnosis. Full-treatment doses of doxycycline or tetracycline should be given. Short "prophylactic" courses of tetracycline or erythromycin are ineffective for eradication of chlamydial infection and may not reliably prevent postabortal morbidity.

The roles for *C. trachomatis* in endometrial infection post partum and after cesarean section remain less well established. In one well-studied case, Cytryn et al.[33] cultured *C. trachomatis* from the peritoneal fluid of a 17-year-old woman with a severe infection after cesarean section. In a prospective study Wager noted delayed (>48 hours to 6 weeks) infectious morbidity in 10 (34%) of 29 women with chlamydial infection delivering vaginally compared with 23 (8%) of 300 uninfected women.[34] In marked contrast, other studies fail to link cervical chlamydial infection with puerperal infection. Prospective studies by Harrison et al.[35, 36] and others[37] show no increase in postpartum infection attributable to *C. trachomatis*. In a case-controlled comparison between women with and without infection after cesarean section, Blanco et al.[38] found no increased carriage of chlamydia among infected mothers. A more recent study by Berenson et al.[39] noted a 20% rate of chlamydial carriage or infection among adolescent women with infectious morbidity after cesarean section, but this was not different from the control teenagers. Although the preponderance of findings did not show that chlamydia was a common cause of postpartum infection, clinicians should be aware that such infections occur occasionally. As in the case described by Cytryn et al.[33] clinical recovery may not occur until specific treatment for *C. trachomatis* is administered. If chlamydiae are recovered from postpartum women, the newborn infant, as well as mother's sexual contacts, should be evaluated and treated.

C. trachomatis infection and pregnancy outcome

Various studies have examined the possible roles of maternal *C. trachomatis* infection in adverse pregnancy outcomes. Suggested complications include PROM, preterm labor and birth, low birth weight (<2500 gm), intrauterine growth retardation, and stillbirth, as well as intrapartum and postpartum maternal and neonatal infectious morbidity.

In a 1982 study Martin et al.[40] prospectively evaluated 268 pregnant women and found that 6.7% had findings of positive *C. trachomatis* infection before 19 weeks' gestation. There was a 33% perinatal mortality rate (three fetal and three neonatal deaths) among infected women, but only one neonatal death occurred among uninfected women. Five (28%) of the 18 infected mothers delivered before 37 weeks' gestation compared with 15 (6%) of the 238 uninfected mothers. This investigation led to calls for studies in which other possible associated factors would be analyzed. Subsequently, in a prospective study of 534 pregnant women, Gravett et al.[41] found that cervical infection with *C. trachomatis* was independently associated with PROM, preterm labor, and low birth weight (odds ratio [OR] 2.7; confi-

Table I. Selected observational studies finding an association between the effect of maternal endocervical *C. trachomatis* infection and some adverse pregnancy outcomes

Author	*(Yr)*	*Study design*	*No. of subjects infected/total studied*	*%*	*Preterm birth*	*Low birth weight (<2500 gm)*	*PROM*	*Other*
Martin et al.[40]	(1982)	Prospective case-control	18/268	7	+	+	NE	Fetal deaths
Martius et al.[42]	(1988)	Case-control	NE	NE	+	NE	NE	—
Gravett et al.[41]	(1986)	Prospective observational	47/534	9	+	+	+	—
Harrison et al.[35]	(1983)	Prospective observational	97/1365	7	–	IgM*	IgM	—
Polk[43] (Johns Hopkins Study Group)	(1989)	Prospective observational	80/120	?	+	–	–	Small for gestational age
Hardy et al.[37]	(1984)	Prospective observational	39/105	37	–	†	NE	—
Berman et al.[36]	(1987)	Prospective observational	251/1152	22 5% IgM*	–	IgM	—	—
Sweet et al.[44]	(1987)	Case-control	270 Infected women	—	IgM	–	IgM	—

Other studies finding absence of association are cited in the text.
NE, Not evaluated; *Ig,* immunoglobulin.
*Presence of anti-*C. trachomatis* immunoglobulin or seroconversion.
†Only in association with trichomoniasis.

dence interval 95% [CI] 1.3 to 5.7; $p = 0.01$).[41] In a case study comparing preterm and term deliveries, Martius et al.[42] found an increased association between chlamydia and preterm birth (OR 5.4; 95% CI 1.3 to 23.4; $p = 0.02$). This finding was unchanged after adjustment for the presence of bacterial vaginosis, lactobacillus, or PROM, which suggests an independent effect of *C. trachomatis*. In the largest prospective study of chlamydial cervical infection during pregnancy that controlled for the presence of *Mycoplasma hominis* and other possible risk factors, Polk et al.[43] found that *C. trachomatis* was significantly associated with preterm birth (OR 1.6; 90% CI 1.01 to 2.5) and intrauterine growth retardation (OR 2.4; 90% CI 1.32 to 4.18). The reason for using 90% rather than 95% CI was not explained. On the other hand, a large number of generally smaller prospective studies fail to show an overall association between maternal chlamydial infection and a poor reproductive outcome.[13, 35-37, 44-50]

Insight into these conflicting results is furnished by several prospective studies demonstrating that PROM, preterm birth, and low birth weight were associated with chlamydial infection only among women with an immunoglobulin M antichlamydial antibody response during pregnancy (Table I). Such findings suggest that women with either recent or more extensive and invasive chlamydial infection, possibly involving the upper reproductive tract, are at increased risk.[35, 36, 44] Harrison et al.[35] noted that a full 24% of chlamydia-positive women (8% of the total) were immunoglobulin M positive.

Additional insight into the pathophysiology of chlamydial infection during pregnancy comes from a prospective study by Hardy et al.,[47] which demonstrated an increase in preterm birth and low birth weight only in the presence of coinfection with *Trichomonas vaginalis*. Possible additive or synergistic effects of cervical vaginal microorganisms or bacterial vaginosis have been demonstrated by others.[37]

Further intriguing findings regarding the role of chlamydia in complications of pregnancy come from several prospective, controlled studies of the treatment of chlamydial infection during pregnancy. Ryan et al.[51] obtained cultures from 11,544 women to determine the presence of *C. trachomatis* at the initial prenatal visit. Twenty-one percent were documented to be infected; approximately one half of these women received treatment over several years. Women who remained untreated (1110) had a significant increase in occurrences of PROM, low birth weight, and decreased perinatal survival compared with either treated women (1323) or women with negative cultures (9111).

In a similar but smaller comparative study, Cohen et al.[52] analyzed the pregnancy outcomes of 244 women successfully treated with erythromycin, 79 chlamydia-positive women who failed to comply with or respond to treatment, and 244 chlamydia-free women. Fre-

Table II. Summary of Centers for Disease Control recommendations for screening and treatment of maternal *C. trachomatis* infection in pregnant women

A.	Who? Pregnant women should undergo diagnostic tests* for *C. trachomatis* *N. gonorrhoeae* Syphilis
B.	When? Initial visit and again in third trimester if judged at increased risk; i.e., <25 yr of age, history of sexually transmitted disease, new (<3 mo) sexual partner or multiple partners
C.	Recommended regimens?†‡ Oral erythromycin base, 500 mg four times daily, for 7 days If poorly tolerated: Oral erythromycin base, 250 mg four times daily, for 14 days or Oral amoxicillin, 500 mg three times daily, for 7 days
D.	Partner: Evaluate and treat if contact within 30 days, if tests unavailable, treat
E.	Tests of cure (by culture) are not recommended unless there is question of patient compliance

Modified from Centers for Disease Control. 1989 Sexually transmitted disease treatment guidelines. MMWR 1989;38 (suppl 8):27-8.

*See text.

†Poor tolerance is treated with supportive care or use alternative regimen.

‡Enteric-coated or sustained-release preparations of erythromycin are also effective but more costly.

quencies of preterm delivery (3% vs 14%, $p = 0.0002$), ruptured membranes (7% vs 20%), preterm labor (4% vs 24%), and small for gestational age newborns (13% vs 25%) were significantly lower among successfully treated patients than in patients considered treatment failures. Successfully treated women had outcomes similar to those of uninfected control mothers. Other complications, including stillbirth and postpartum endometritis, were similar among all three groups.

In the only double-blind, randomized, placebo-controlled trial of the treatment of chlamydia during pregnancy,[53] 125 infected pregnant women enrolled before 24 weeks' gestation were treated with either erythromycin, clindamycin, or placebo. No differences in effectiveness between clindamycin or erythromycin were noted. Although patients with preterm PROM were more likely to have been infected with *C. trachomatis* (or trichomoniasis), eradication of *C. trachomatis* before the third trimester failed to reduce significantly the occurrences of PROM, preterm birth, low birth weight, or maternal infectious morbidity in this small but well-controlled study.

Such inconsistent findings are not necessarily unexpected when one considers variations in study size, appropriateness of controls, prevalence of infection, type of microbiologic diagnosis, serologic assessment, and attempts to control for confounding factors. Several large-scale, controlled, prospective studies of erythromycin treatment during pregnancy are in progress and should furnish further information on chlamydia-associated pregnancy morbidity as well as the effects of treatment. Furthermore, metaanalysis may be used to aggregate the findings of comparable studies and may reveal new information regarding the roles of chlamydial infection of the reproductive tract and various populations.

Diagnosis

The methodology of both culture and nonculture microbiologic tests for *C. trachomatis* is not altered during pregnancy.[53, 54] Careful specimen collection, handling, and processing are important for optimal diagnosis. In both pregnant and nonpregnant women, chlamydial cultures are 100% specific and approximately 80% to 90% sensitive.[53, 54]

In a population of pregnant women with a relatively high prevalence of infection (21.5%), sensitivities and specificities for a direct fluorescent antibody test were 98% and 95%, respectively.[55] Similar results of 96% and 93%, respectively, were also noted for an enzyme immunoassay test.[54] In groups of women with lower prevalences of chlamydial infection, culture techniques may be considerably more accurate.

Alternative methods such as deoxyribonucleic acid hybridization and polymerase chain reaction are being developed.[56, 57] Anecdotal information suggests that identification of presumed chlamydial infection by examination of cervical mucin for white blood cells or discoloration is less accurate in pregnant than in nonpregnant women.

Ongoing advances in culture, antigen detection, and molecular biologic techniques will permit less costly but equally accurate diagnosis of chlamydial infection.

Present recommendations for screening and treatment

Although the effects of antichlamydial therapy on pregnancy outcomes, such as preterm birth, PROM, and postpartum endometritis, remain uncertain, the benefits of identifying and treating infection during pregnancy to prevent neonatal infection and morbidity are increasingly clear. Postpartum topically applied chemoprophylaxis should be retained, although it provides incomplete protection. Topical application of erythromycin or tetracycline to prevent conjunctivitis has not prevented pneumonia and subsequent respiratory disease, because conjunctival prophylaxis does not eliminate colonization of the respiratory tract. Recent studies have confirmed Bell's original suggestions that topical antibiotic prophylaxis is no more effective than the less expensive 1% silver nitrate fluid prophylaxis.[28]

To prevent neonatal morbidity, identification and eradication of maternal chlamydial infection during pregnancy are required. A large prospective, nonrandomized study by Schachter et al.[58] and smaller studies by FitzSimmons et al.,[46] Black-Payne et al.,[59] and McMillan et al.[60] demonstrate that the diagnosis of chlamydia by culture or antigen-based techniques permits effective treatment with erythromycin and successful prevention of chlamydia-associated morbidity in newborn infants.

Schachter et al.[58] offered treatment with oral erythromycin ethylsuccinate, 400 mg four times a day, for 7 days at 36 weeks' gestation to 184 pregnant women with cervical chlamydial infection. Thirty-two women refused treatment and served as control subjects. Of the 152 women who took the medication, 10 complained of gastrointestinal disturbances, and erythromycin had to be discontinued in 5 women. Of those who completed the protocol, 92% had negative findings of chlamydia. Chlamydial infection or colonization occurred in only 7% of the newborn infants of treated mothers compared with 50% of infants born of untreated women.

FitzSimmons et al.[46] followed 33 women infected with cervical chlamydia during the first half of pregnancy and started treatment at 36 weeks' gestation. None of the infants of the 18 women who received adequate treatment with erythromycin had infection or chlamydial-linked morbidity. Conversely, two of the three newborn infants of untreated women had positive findings of chlamydia. Treatment at 36 weeks does not allow for prophylaxis for women delivering preterm or for women who were away during the course of care.

Black-Payne et al.[59] used an enzymatic immunoassay test for the diagnosis of chlamydial cervicitis between weeks 28 and 32 of pregnancy and provided treatment with oral erythromycin. Although 16% of their patients noted side effects, 92% completed therapy, with apparent elimination of excess chlamydial-associated morbidity. The double-blind, randomized trial of Alger and Lovchik[53] showed that oral erythromycin or clindamycin was as well tolerated as placebo.

Although these studies did not demonstrate high rates of intolerance to erythromycin during pregnancy, anecdotal reports from several institutions and our observations[49] suggest that a substantial number of pregnant women do not complete erythromycin therapy because of adverse gastrointestinal effects. In such cases, reduced doses of erythromycin or use of agents such as clindamycin and amoxicillin may be better tolerated.[61, 62] Both clindamycin and amoxicillin appear effective and may offer real advantages for the treatment of chlamydial infection during pregnancy.[61-63]

The Centers for Disease Control has recently issued new recommendations regarding screening and treatment of *C. trachomatis* during pregnancy (Table II).[62] At this time, treatment late in pregnancy is recommended to prevent perinatal infection caused by maternal reinfection or relapse. Alternatively, screening and treatment may be completed early in pregnancy, with reculture and treatment near term in selected women. In either instance sexual partners should be sought, evaluated, and treated for chlamydia. Similarly, both parents of chlamydia-infected infants should be evaluated and treated.

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New treatments for *Chlamydia trachomatis*

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Standard regimens of tetracycline, doxycycline, or erythromycin, if complied with, appear to be effective against *Chlamydia trachomatis* infections under most circumstances. However, the organism may sometimes persist despite what would seem to be adequate therapy. How often this occurs, to what extent noncompliance is the issue, and the role antibiotic resistance plays remain to be determined. Among newer antibiotics, azithromycin appears to be effective in the treatment of uncomplicated urogenital *C. trachomatis* infections. Single-dose therapy with azithromycin may be especially useful in overcoming compliance problems associated with the treatment of sexually transmitted disease. (Am J Obstet Gynecol 1991;164:1789-93.)

Key words: *C. trachomatis*, recurrent infection, antibiotics, azithromycin

Chlamydia trachomatis infections of the genital tract are prevalent among young, sexually active persons. They are associated with considerable morbidity, including infertility, ectopic pregnancy, infant pneumonia, and conjunctivitis.[1,2] Efforts at controlling transmission and preventing complications have focused on the identification of infected persons and treatment with effective antibiotics. Selection of the antibiotic has been based primarily on demonstrated efficacy in clinical trials and on cost.[3,4]

The treatment presently recommended for uncomplicated genital tract infection with *C. trachomatis* is doxycycline, 100 mg orally twice a day for 7 days, or tetracycline, 500 mg orally 4 times a day for 7 days.[5] The essential therapeutic equivalency of tetracycline, doxycycline, and minocycline has been established in several clinical trials.[4] Doxycycline has become the preferred agent, because less frequent dosing is required.[5] Minocycline has been used less often because of vestibular side effecs, particularly at higher doses.

Of interest has been the observations that clindamycin in combination with an aminoglycoside has been effective in eradicating *C. trachomatis* in women with salpingitis,[6] whereas clindamycin alone was ineffective in treating men with chlamydial urethritis.[7] A possible explanation may lie in the recent demonstration of in vitro synergy of clindamycin and gentamicin or tobramycin against *C. trachomatis* despite the fact that the aminoglycosides are inactive when used alone.[8]

Recurrent or persistent infections

Although the present treatment regimens appear effective in most patients who are compliant, there has been a failure rate of approximately 5% in most studies in which a test-of-cure culture is obtained 4 to 7 days

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Table I. Time to recurrence of *C. trachomatis* infection in adolescent girls

Mo after initial infection	*Recurrence rate*	
	No. positive/No. tested	*%*
0-3	4/34*	12
3-6	20/79	25
6-9	13/34	38
9-12	13/34	38
12-15	4/20	20
15-18	7/13	54
18-21	4/7	57
21-24	3/5	60
Cumulative	68/176	39

*Test of cure visits not included. Each subject counted only at the time of first recurrence, if she had more than one.

after completion of therapy.[4] Furthermore, a high proportion of persons with chlamydial infections who come to a sexually transmitted diseases clinic have recurrent infections.[9] In a study performed in Indianapolis between Sept. 1, 1983, and April 15, 1986, 5722 persons attending a sexually transmitted diseases clinic were identified by culture as having a chlamydial infection. Of these, 2224 (39%) returned at least once during the same period because of symptoms or concern about having a sexually transmitted disease, and 573 (26%) were again infected with *C. trachomatis*—some on as many as five separate occasions.[9]

These observations led to a closer evaluation of recurrent chlamydial infection, particularly in the setting of concurrent gonococcal infection at the time of recurrence. It was observed that a high proportion of persons (47%) with concurrent gonococcal infection were again infected with the same serovar of *C. trachomatis.*[3] In patients with no gonococcal infection, recurrence of the same serovar was considerably less frequent (22%). This association between gonococcal infection and the same serovar recurrence persisted for more than a year. By contrast, in persons without gonococcal infection, same serovar recurrence was infrequent beyond 6 months.[9]

The most likely explanation of these findings is that a number of patients had not been cured of the original chlamydia infection, which was somehow reactivated by the gonococcal infection. In patients without gonorrhea, the occurrence of same serovar reinfections at less than 6 months may represent either relapses or reinfections from untreated partners. In this group, however, most reinfections after more than 6 months involve a different serovar and presumably represent a new exogenous infection. If the original serovar was still present at the time of the second infection, it would have been replaced by the newly infecting serovar, perhaps by further stimulation of the immune response.

Preliminary data from another study in adolescent girls also indicate a high rate of recurrence.[10] Adolescent girls attending county health department clinics, primarily for contraception, pregnancy testing, and other gynecologic services, were cultured for *C. trachomatis* and followed up, respectively. One hundred seventy-six girls with positive cultures had a negative culture result within 3 months after treatment and had a second culture taken at a subsequent return visit, which was usually for contraceptive purposes (Table I). At each evaluation after the first 3 months, a recurrence rate greater than 20% was observed, with a cumulative rate of 39% for the entire 2-year follow-up period.

Distinguishing relapse from same serovar reinfection is particularly difficult in this population. None of the women was abstinent during the interval between infections, and many resumed sexual activity with a former partner who had not been treated for a chlamydial infection. Nevertheless, these data suggest that little if any protection against reinfection results from a natural infection and that persistence may represent more of a problem than is generally recognized.

Other evidence supporting such a concept includes the documentation of persistent *C. trachomatis* infection in infants for as long as 2 years,[11] as well as persistent infection with lymphogranuloma venereum strains for as long as 20 years in persons not likely to have been reinfected.[12]

In addition, there is an animal model in which persistence of sexually transmitted chlamydia infection is observed. Guinea pigs infected intraurethrally with low doses of the guinea pig inclusion conjunctivitis agent may have no evidence of infection and become culture negative. Yet they remain capable of transmitting the agent to other guinea pigs.[13]

Infertility and antibiotic resistance

Persistent infection may also be a contributing factor to infertility in women. We recently reported the recovery of *C. trachomatis* from the fallopian tubes and endometrium of 8 of 52 women (15%) undergoing microtuboplasty for tubal infertility.[14] In two women who had received tetracycline before microtuboplasty, the organisms were relatively resistant to tetracycline in vitro.[15] These isolates were also resistant to erythromycin, clindamycin, and sulfamethoxazole but were sensitive to ciprofloxacin, ofloxacin, and rifampin. One of the patients was treated with minocycline after microtuboplasty. She had a culture negative result in the lower genital tract and conceived. The other patient was lost to follow-up.

Table II. Eradication of *C. trachomatis* by β-lactam antibiotics and erythromycin

		Bacteriologic cure rate		
Site infected, reference	*Antibiotic*	*Day 7**	*Day 14*	*%*
Urethra (F)/cervix	Pivampicillin, 700 mg 2 times a day for 7 days	26/26	26/26	19
	Erythromycin, 500 mg 2 times a day for 7 days	23/23	22/23	
Urethra (M)	Amoxicillin, 750 mg 3 times a day for 7 days		11/11	20
Cervix	Ampicillin, 3.5 gm loading dose, then 500 mg 4 times a day for 7 days		10/12	21
Cervix (postpartum)	Timentin (5), piperacillin (2)		6/7†	22
Cervix (postpartum)	Cefoxitin (2), cefotetan (1)		0/3	
Endometrium (Salpingitis)	Cephalosporin		2/10‡	23

*After initiation of therapy.
†Three to 58 days after completion of therapy.
‡Type of follow-up cultures not specified.

The clinical significance of the type of resistance observed in these isolates remains to be determined. It was heterotypic, because only a small proportion of organisms in each isolate showed high-level resistance to tetracycline after in vitro growth.[15] Consequently, both a high inoculum and immunofluorescent staining of inclusions were necessary to demonstrate resistance. In addition, the resistant isolates did not grow well in vitro and may have had limited in vivo viability.

Erythromycin and β-lactam antibiotics

Treatment of chlamydial infections in pregnancy has proved to be a particularly difficult problem. Tetracycline cannot be used, and many pregnant women cannot tolerate the gastrointestinal side effects associated with erythromycin.

However, in one study in which oral erythromycin ethylsuccinate was given to infected pregnant women at a dose of 400 mg four times a day, cure rates of 92% were observed. Only 3% of subjects discontinued medication because of gastrointestinal intolerance.[16]

In a subsequent study in pregnant women, oral amoxicillin, 500 mg three times a day for 7 days, was compared with an oral erythromycin base, four times a day for 7 days. There was a slightly higher cure rate in the amoxicillin-treated women, and the regimen was better tolerated.[17] In a much smaller study nine infected, pregnant women were treated with comparable doses of amoxicillin for 10 days, and three were not cured.[18] Thus although further evaluation is in order, amoxicillin appears to be an acceptable alternative to erythromycin when erythromycin cannot be tolerated.

Limited data indicate that when given for several days, penicillins or erythromycin may also be effective for the treatment of chlamydial infections in other situations (Table II).[19-23] However, to date, single-dose or short-course therapy has been ineffective,[20] and cephalosporins have not been effective treatment when tried in patients.[23]

Table III. In vitro activity of selected antimicrobial agents against *C. trachomatis*

Antimicrobial agent	*MIC range (μg/ml)*
Tetracycline	0.02-0.5
Doxycycline	0.03-0.05
Erythromycin	0.05-0.5
Clindamycin	0.25-2.0
Azithromycin	0.26-1.02
Ampicillin	0.2-50
Sulfamethoxazole	4-50
Rifampin	0.005-0.25
Ofloxacin	0.5-2.0
Ciprofloxacin	1.0-4.0
Fleroxacin	1.5-2.5
Lomefloxacin	2.0-4.0
Perfloxacin	2.0-8.0
Norfloxacin	8.0-16.0
Enoxacin	8.0-16.0

See references 24 through 28.
MIC, Minimum inhibitory concentration.

New antimicrobials

Some of the fluoroquinolones and the azalide antibiotic, azithromycin, demonstrate significant promise for the treatment of chlamydial infections. Table III outlines the in vitro susceptibility of *C. trachomatis* to several of these agents.[24-28]

Ciprofloxacin was one of the first fluoroquinolones to undergo clinical trials for the treatment of chlamydial infections.[29, 30] Despite excellent in vitro activity, a substantial incidence of late failures was observed when patients had cultures taken 2 or more weeks after completion of therapy. Norfloxacin is ineffective in the treatment of chlamydial infections.[25] Ofloxacin demonstrated in vitro activity comparable with that of ciprofloxacin; however, therapy with a 7-day course of this drug resulted in a 99% cure rate, which is comparable with that of a full course of doxycycline.[31]

Because of the prolonged chlamydial life cycle of 48 to 72 hours, effective antibiotic treatment requires therapeutic levels of a drug at the intracellular site of infection for sustained periods of time. Standard treatment regimens entail multiple daily doses of antibiotic for at least 7 days. Compliance is often poor and recurrence remains a problem. Achieving medical compliance in the sexual partner of the index patient is an additional challenge to eradicating the chlamydial infection.

Azithromycin concentrates extensively within cells and has a tissue half-life of approximately 60 hours.[32] Azithromycin levels in human tissues after a single 500 mg dose may exceed the minimum inhibitory concentration of *C. trachomatis* by threefold to 10-fold for as long as 5 days. Clinical trials are examining the efficacy of twice that dose, 1 gm, for the treatment of chlamydial urethritis and cervicitis. If effective, a single-dose regimen with azithromycin would ensure adequate therapy and eliminate the compliance problem.

In two published studies, one conducted in Iceland[33] and the other in Finland,[34] a single 1 gm dose of oral azithromycin was as effective as 100 mg of doxycycline given twice a day for 7 days in eradicating urogenital *C. trachomatis* infections in both men and women. Side effects, primarily gastrointestinal, were mild and of similar incidence in the doxycycline and azithromycin groups. Additional data from two multicenter European studies seem to confirm these observations.[35]

In summary, new approaches to the treatment of chlamydial cervicitis and urethritis include compounds from the azalide and fluoroquinolone classes. Azithromycin, the first of the azalide antibiotics, offers the promise of efficacious single-dose therapy, an advance that should significantly improve control of this pathogen and help in the prevention of its serious sequelae.

Summary

Standard tetracycline, doxycycline, or erythromycin therapy appears to be effective against *C. trachomatis* infection under most circumstances. Doxycycline has been the preferred treatment because less frequent administration is required.

Nevertheless, test-of-cure cultures obtained 4 to 7 days after completion of standard therapy indicate a failure rate of approximately 5%. Frequent recurrence of the same chlamydial serovar in patients with gonococcal infections also suggests that the original infection may not have been cured.

A new azalide antibiotic, azithromycin, has been undergoing intensive clinical investigation as single-dose therapy for chlamydial urethritis and cervicitis. A single 1 gm dose has shown efficacy comparable with a standard 7-day course of doxycycline. The side effect profile has been mild, with an incidence similar to that of doxycycline. Effective single-dose therapy of chlamydial infections with azithromycin will significantly reduce compliance problems and enable greater control of chlamydia in the general population. It is hoped that this will ultimately reduce the serious sequelae caused by the pathogen.

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The role of azalide antibiotics in the treatment of chlamydia

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Chlamydia trachomatis causes an estimated 4 million infections each year in the United States. Sequelae in women include infertility and ectopic pregnancy. Doxycycline, the current standard therapy, must be taken twice daily for at least 7 days. Patient compliance is often poor, leading to recurrent disease. Azithromycin, a new antibiotic of the azalide class, achieves high and sustained intracellular levels and demonstrates excellent in vitro activity against *C. trachomatis*. Reported herein are the results of three comparative clinical trials, which demonstrate that single-dose oral therapy with azithromycin is as effective as a standard 7-day twice-daily course of doxycycline in the treatment of uncomplicated genital infections caused by *C. trachomatis*. (AM J OBSTET GYNECOL 1991;164:1794-6.)

Key words: Chlamydia, gonorrhea, doxycycline, azithromycin

Chlamydial infection is the most frequently encountered sexually transmitted disease in developed countries today, with an estimated 4 million infections each year in the United States alone.[1, 2] *Chlamydia trachomatis* is a bacterium that infects humans as an obligate intracellular parasite. Its life cycle is prolonged and is characterized by serial transformation between infectious and replicative forms. The genital serovars of *C. trachomatis* can cause devastating medical complications. Chlamydial infection of the cervix may ascend within the female genital tract and result in endometritis or salpingitis. This, in turn, may lead to tubal scarring, with resultant ectopic pregnancy or infertility. A newborn infant exposed to chlamydia in the birth canal may develop conjunctivitis or pneumonitis.[1] In men, *C. trachomatis* is a cause of urethritis, epididymitis, and proctitis.

Whereas urethritis and cervicitis caused by *Neisseria gonorrhoeae* are readily treated with single-dose regimens, standard therapy for *C. trachomatis* is problematic. The Centers for Disease Control currently recommends oral treatment of uncomplicated urethral, endocervical, and rectal infections caused by *C. trachomatis* with either doxycycline, 100 mg twice daily for 7 days, or tetracycline, 500 mg four times daily, for the same period of time. The inconvenience of multiple-dose therapy with doxycycline or tetracycline may lead the patient to discontinue therapy prematurely, with the possibility of resultant treatment failure. Because of this problem, there is a strong need for a single-dose therapy of *C. trachomatis* that is safe, effective, and well tolerated.[3-5]

Azithromycin: Spectrum and pharmacokinetics

Azithromycin is the prototype azalide antibiotic chemically related to erythromycin. It differs from that agent by virtue of its microbiologic spectrum, tolerability, and completely novel pharmacokinetics. Important sexually transmitted pathogens in the spectrum of azithromycin include *C. trachomatis*, *N. gonorrhoeae*, *Treponema pallidum*, and *Haemophilus ducreyi*, the causative agent of chancroid.

The pharmacokinetics of azithromycin are unique. It is avidly taken up by cells, resulting in high and sustained concentrations in tissues. A natural drug delivery system occurs as migratory leukocytes loaded with azithromycin are carried to sites of inflammation, further increasing local drug concentration.[6]

Azithromycin is acid stable, and bioavailability after a single 500 mg oral dose is 37%. The peak serum level after this dose is only 0.4 μg/ml; however, concentrations within tissues and cells are from 10- to 50-fold higher than in serum (Fig. 1). After oral administration, serum concentrations of azithromycin decline in a polyphasic pattern, resulting in an average terminal half-life of 68 hours. The high values for apparent steady-state volume of distribution (31.1 L/kg) and plasma clearance (630 ml/min) suggest that the prolonged half-life is from extensive uptake and subsequent release of drug from tissues. Excellent drug levels are attained in the ovary, fallopian tubes, uterus, and cervix (Table I).[7] Tissue levels of azithromycin are well in excess of the minimal inhibitory concentrations of most commonly encountered pathogens, and tentative guidelines suggest that susceptible organisms be considered those with minimal inhibitory concentrations of 90% that are less than or equal to 2.0 μg/ml.[8]

Clinical experience with azithromycin

The high and prolonged tissue concentrations of azithromycin allow its administration on a once-daily basis, with a shorter course of therapy (1 to 5 days) than

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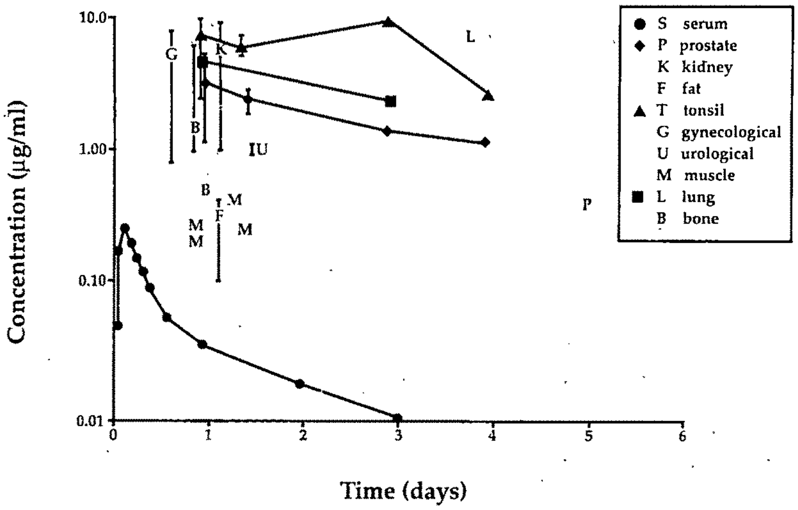

Fig. 1. Human serum and tissue concentrations after administration of 500 mg of azithromycin.

with standard agents. These unique pharmacokinetics, in conjunction with the excellent in vitro efficacy against *C. trachomatis*, suggested exploration of the efficacy of short-course oral therapy with azithromycin in the treatment of uncomplicated genital infections caused by chlamydia. The results of three trials performed in Europe, which involved more than 500 patients, are presented. All studies compared azithromycin therapy, administered as one to three doses, with 7-day twice-daily therapy with doxycycline. Tests for *C. trachomatis* were performed by culture in McCoy cells on swab specimens obtained from the urethra or endocervix. Positive cultures were identified when monoclonal antibodies bound successfully to chlamydial inclusions.

Single-dose efficacy. The first study* was designed to assess the safety and antichlamydial efficacy of a single 1 gm dose of azithromycin compared with a standard regimen of doxycycline, 100 mg twice daily for 7 days. One hundred forty-eight patients from 12 centers were treated with azithromycin, and 143 were treated with doxycycline. Eradication of *C. trachomatis* in evaluable patients was assessed 1 and 2 weeks after the initiation of therapy. At 1 week, 61 of 66 patients (92%) treated with azithromycin had eradication of the pathogen compared with 36 of 39 patients (92%) treated with doxycycline. At 2 weeks, the eradication rates for azithromycin and doxycycline were 96% (49/51) and 95% (36/38), respectively. Side effects were of mild to moderate severity, mostly related to the gastrointestinal tract, and were comparable for both agents (Table II). This study provided the first evidence that a single-dose regimen of azithromycin was comparable with a 7-day (14-dose) regimen of doxycycline in the treatment of uncomplicated genital chlamydial infections.

Table I. Azithromycin concentrations in human tissues 17 hours after oral administration of 500 mg

Tissue type	*No. of gynecologic tissues*	*Mean tissue concentration (µg/gm)*
Fallopian tube	5	3.3
Ovary	5	2.7
Uterus	4	3.5
Cervix	3	2.8
Fibroid tissue	1	2.2
Peritoneal adhesion	1	4.0
Serum	7	0.036
Peritoneal fluid	7	0.078

Table II. Results of study 1: Single-dose azithromycin*

	Azithromycin		*Doxycycline*	
Time period	*Total response (%)*	*Female response (%)*	*Total response (%)*	*Female response (%)*
Wk 1	61/66 (92)	28/29 (97)	36/39 (92)	23/24 (96)
Wk 2	49/51 (96)	22/23 (96)	36/38 (95)	22/23 (96)

*Treatment-related side effects: azithromycin, 35 of 148 (24%); doxycycline, 29 of 146 (20%). Most were of mild to moderate severity of the gastrointestinal tract.

A second study† examined the safety and efficacy

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*Investigators in study 1 included the following: Professor P. M. Carsten, Germany; Dr. M. Dubois, Belgium; Dr. J. B. Garrel, France; Dr. A. Hallen, Sweden; Dr. E. Kutzer, Germany; Dr. J. G. Lauritsen, Denmark; Dr. K. Lind, Sweden; Professor P. Morel, France; Dr. P. Normand, France; Dr. R. Oitnner, Germany; Dr. F. H. F. Plantema, The Netherlands; and Dr. F. Verbeeck, Belgium.

†Investigators in study 2 included the following: Drs. E. T. Houang and A. G. Lawrence, United Kingdom; Dr. K. Krohn, Sweden; Professor A. Lassus, Finland; Dr. R. N. T. Thin, United Kingdom; Dr. S. Ullman, Denmark; and Dr. K. Weisman, Denmark.

Table III. Results of study 2: Two regimens of azithromycin*

	Azithromycin		*Doxycycline*	
Time period	*Single dose*	*3 Days*	*200 mg day/100 mg × 6 days*	*200 mg/day*
Wk 1	34/34	43/43	2/2	36/38 (95%)
Wk 2	34/35 (97%)	30/31 (97%)	6/6	27/28 (96%)

*Treatment-related side effects: azithromycin, 15/196 (8%); doxycycline, 6/96 (6%). All were of mild to moderate severity of the gastrointestinal tract, and no treatment was discontinued because of side effects.

Table IV. Results of 4-week follow-up study*

	Azithromycin			*Doxycycline*
Time period	*Single dose*	*1 Day*	*3 Days*	*200 mg/day × 7 days*
Wk 1	72/73	20/20	20/20	63/64 (98%)
Wk 2	68/70 (97%)	22/22	20/20	59/59
Wk 4	62/64 (97%)	17/18 (94%)	19/19	58/58

*Treatment-related side effects: azithromycin, 18/164 (11%); doxycycline, 11/83 (13%). Most were of mild to moderate severity of the gastrointestinal tract, and no treatment was discontinued because of side effects.

1 gm of azithromycin, administered either as a single dose or in divided doses over 3 days (500 mg on day 1, followed by 250 mg on 2 successive days). Control groups were treated with doxycycline, 100 mg twice daily for 7 days, or 200 mg on day 1, followed by 100 mg daily for 6 additional days. Data on the eradication of chlamydia at the 1- and 2-week assessments are presented in Table III. As can be seen, eradication rates in all groups were comparable at the 2-week follow-up visit. In addition, the safety profiles were also comparable.

Eradication of chlamydia: Four-week follow-up. An additional study was performed by Dr. O. Steingrimsson from Iceland to assess eradication of *C. trachomatis* at 1, 2, and 4 weeks after therapy with azithromycin or doxycycline. Azithromycin was administered in three different regimens for either 1 or 3 days (1 gm orally for 1 dose; 500 mg orally every 12 hours on 1 day; or 500 mg orally on day 1, followed by 250 mg daily for the next 2 days). Control patients were treated with oral doxycycline 100 mg twice-daily for 7 days. All regimens showed comparable efficacy (Table IV). Effective eradication was confirmed by 1-, 2-, and 4-week follow-up cultures. Again, the safety profiles were comparable.

Clinical trials have shown that a single 1 gm dose of azithromycin is safe and effective in the treatment of uncomplicated genital infections caused by *C. trachomatis*. Results compared favorably with multiple-dose therapy with doxycycline, the current "standard therapy." Patient compliance with multiple-dose regimens is a major concern in the treatment of sexually transmitted diseases.[9] The convenience and efficacy of single-dose therapy with azithromycin are expected to provide significant benefit in the clinic, because compliance with therapy is virtually assured.

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